Université de Montréal

Rôles des modifications de la microflore bactérienne et de l'exsudation racinaire de la tomate par la symbiose mycorhizienne dans le biocontrôle sur le *Phytophthora nicotianae*

par

Laetitia Lioussanne

Département de Sciences Biologiques
Institut de recherche en biologie végétale
Ecole Polytechnique de Montréal

Faculté des Arts et des Sciences

Thèse présentée à la Faculté des études supérieures
en vue de l'obtention du grade de Philosophiæ Doctor (Ph.D.) en sciences biologiques

avril, 2007

Université de Montréal
Faculté des études supérieures

Cette thèse intitulée :

Rôles des modifications de la microflore bactérienne
et de l'exsudation racinaire de la tomate par la symbiose mycorhizienne
dans le biocontrôle sur le *Phytophthora nicotianae*

présentée par :
Laetitia Lioussanne

a été évaluée par un jury composé des personnes suivantes :

Roxane Maranger, président-rapporteur
Marc St. Arnaud, directeur de recherche
Mario Jolicoeur, co-directeur
Mohamed Hijri, membre du jury
John Larsen, examinateur externe
, représentant du doyen de la FES

Résumé

Les champignons mycorhiziens à arbuscules (CMA), en symbiose mutualiste avec la majorité des plantes terrestres, favorisent les nutritions minérale et hydrique et augmentent la résistance aux stress abiotiques et biotiques des végétaux. Les CMA induisent ainsi un biocontrôle caractérisé par la réduction des symptômes des maladies provoquées principalement par les champignons, stramenopiles et nématodes phytopathogènes du sol. Nous avons confirmé, lors de culture en sol, que le CMA *Glomus mosseae* (Nicol. et Gerd.) Gerdemann et Trappe réduisait la prolifération du *Phytophthora nicotianae* Breda de Haan dans les racines de tomate et obtenu, pour la première fois, des résultats similaires lors de l'inoculation du *G. intraradices* Schenck et Smith (DAOM 181 602). L'attraction massive des zoospores (libérées par reproduction asexuée du *P. nicotianae*) par les exsudats racinaires est une étape cruciale nécessaire à la prolifération de ce stramenopile phytopathogène du sol dans les tissus hôtes. Nous avons montré, *in vitro*, que les exsudats de racines de tomate matures, transformées par l'*Agrobacterium rhizogenes* et colonisées par le *G. intraradices* repoussent les zoospores du *P. nicotianae* et contiennent significativement davantage de proline que les exsudats de racines non colonisées et/ou plus jeunes. Afin de valider l'implication de la modification de l'exsudation racinaire dans le biocontrôle induit par les CMA, des plants non-transformés ont été cultivés *in vivo*. L'application d'exsudats de racines colonisées par le *G. intraradices* ou le *G. mosseae* sur des racines de plants de tomate cultivés en sol n'a pas réduit la biomasse intraracinaire du *P. nicotianae* de façon similaire à l'inoculation directe de spores de ces CMA. Par PCR-DGGE du gène

16S ribosomal, nous avons montré que la colonisation racinaire de tomate par ces CMA modifiait significativement la communauté bactérienne de la rhizosphère de tomate, contrairement à l'application d'exsudats de racines colonisées par ces CMA et à l'inocluation du *P. nicotianae*. La modification de la communauté bactérienne de la rhizosphère induite par les CMA nécessiterait leur présence physique. La modification de l'exsudation racinaire par les CMA ne réduirait pas significativement la prolifération du *P. nicotianae* dans les tissus hôtes de façon directe ou indirecte (par modification de la communauté bactérienne du sol). Trente-quatre variants de séquence différents du gène ribosomal 16S ont été observés par PCR-DGGE (réalisé par amplification de l'ADN extrait directement des spores) alors que dix-huit isolats bactériens (classés selon neuf groupes phylogénétiquement distincts appartenant aux trois genres : *Paenibacillus*, *Bacillus* et *Methylobacterium*) ont été obtenus par incubation sur un milieu de culture standard, à partir de spores du *G. mosseae* désinfectées en surface. Les isolats identifiés en tant que *B. simplex*, *B. niacini*, *B. drententis*, *Paenibacillus* spp. et *Bacillus* sp. se sont avérés, *in vitro*, antagonistes envers différents agents pathogènes du sol, surtout vis-à-vis du *P. nicotianae*. Ainsi, les bactéries associées aux structures des CMA seraient impliquées dans le biocontrôle qu'ils induisent sur le *P. nicotianae*, chez la tomate.

Mots Clés

Glomus mosseae, *G. intraradices*, zoospores, chémotaxie, exsudats racinaires, DGGE, endobactéries, mycorhizosphère, agents pathogènes du sol.

Abstract

Arbuscular mycorrhizal fungi (AMF) form a mutualistic symbiosis with most land plants and have been shown to favour their mineral and water nutrition and to increase their resistance to abiotic and biotic stresses leading to the apparition of bioprotection (or biocontrol). This phenomenon is characterized by the reduction of disease symptoms provoked mainly by soilborne phytopathogens such as fungi, stramenopiles and nematodes. We confirmed that the AMF *Glomus mosseae* (Nicol. et Gerd.) Gerdemann et Trappe (BEG 12) reduced the proliferation of *Phytophthora nicotianae* Breda de Haan within tomato roots and obtained, for the first time, the same results by the inoculation of *G. intraradices* Schenck et Smith (DAOM 181 602). The massive attractiveness by root exudates to zoospores (liberated through the asexual reproduction of *P. nicotianae*) is a crucial step necessary for the host tissue invasion by this soilborne stramenopile. We showed, *in vitro*, that exudates from mature tomato roots transformed by *Agrobacterium rhizogenes* and colonized with *G. intraradices* were repulsive among the zoospores of *P. nicotianae* and contained significantly more proline than non-colonized and/or younger transformed tomato roots. In order to validate the implication of root exudation modification after mycorrhizal colonization in biocontrol induced by AMF, non-transformed plants were grown *in vivo*. The application of exudates from tomato roots colonized with *G. intraradices* or with *G. mosseae* on tomato roots grown in soil, did not reduce the *P. nicotianae* biomass within root tissues to the same extent as did the direct root colonization with these AMF. Using molecular profiling of the bacterial population by PCR-DGGE, from the 16S rRNA gene, we showed that root

colonization with these AMF significantly modified the bacterial community of the tomato rhizosphere whereas the application of exudates from mycorrhizal roots or the inoculation of *P. nicotianae* did not. The presence of mycorrhizal structures would be necessary for the modification of the bacterial community that AMF induce on the rhizosphere. Root exudation modification induced by AMF colonization would not significantly modify *P. nicotianae* proliferation within host tissues directly (by inhibiting its attraction from the soil) or indirectly (through rhizosphere bacterial community changes). Thirty-four different sequence variants of the 16S rRNA gene were observed after a PCR-DGGE (performed by the amplification of the DNA directly extracted from the spores) whereas eighteen bacterial isolates (classified into nine phylogenetically distinct groups belonging to three genera: *Methylobacterium*, *Bacillus* and *Paenibacillus*) were isolated from a standard culture medium, from surface disinfected spores of *G. mosseae*. *In vitro*, fourteen culturable isolates, especially isolates identified as *B. simplex* but also as *B. niacini*, *B. drententis*, *Paenibacillus* sp. and *Methylobacterium* sp. showed antagonism among different soilborne pathogens, especially over *P. nicotianae*. Bacteria associated withAMF structures would be implicated in the biocontrol AMF induce on *P. nicotianae*, in tomato plants.

Keywords

Glomus mosseae, Glomus intraradices, soilborne pathogens, root exudates, zoospores, chemotaxy, DGGE, endobacteria, mycorrhizosphere.

Table des matières

Liste des Tableaux

Liste des Figures

Liste des sigles et des abréviations

Ø :	Diamètre
Ac. :	Acide
ADN :	Acide désoxyriblonucléique (DNA)
AJ :	Acide jasmonique (JA)
Al :	Aluminium
Ala :	Alanine
Arg :	Arginine
Asn :	Asparagine
Asp :	Aspartate
AMF :	Arbuscular mycorrhizal fungus
ARN :	Acide ribonucléique (RNA)
ANOVA :	Analysis de variance
ATCC :	American type culture collection
BEG :	Banque européenne des glomales
Blast :	Basic local alignment search tool
BSA :	Bovine serum albumin
C :	Carbone
Ca:	Calcium
CANDISC :	Canonical discriminent analysis
cf. :	*Confer*
Cl :	Chlorure
cm :	centimètre
CMA :	Champignon mycorhizien à arbuscules
Ca :	Calcium
CO_3 :	Carbonate
CORRESP:	Correspondence analysis of contingency
CP :	Composante principale
CLHP :	Chromatographie en phase liquide de haute performance (HPLC)
CRSNG :	Conseil de recherche en sciences naturelles et en génie du Canada (NSERC)
DAOM :	Département d'agriculture en mycologie, Ottawa
DGGE :	Gel d'électrophorèse en gradient dénaturant
DI :	Diamètre d'inhibition
DMSO :	Dimethyl sulfoxide
DNTPs :	Désoxyribonucléotides tri-phosphate
DO :	Densité optique (OD)
Dr. :	Docteur
DRB :	Deleterious Rhizobacteria
E+ :	Application d'exsudats racinaires de tomate
E- :	Application d'eau pure stérilisée
ELISA :	Enzyme Linked ImmunoSorbent Assay
EPS :	Polysaccharides extracellulaires

et al. **:**	*Et alia*
f. sp. :	*Formae speciales*
g :	Gramme
FQRNT:	Fonds québécois de la recherche sur la nature et la technologie
Fe :	Fer
Fig :	Figure
Fruc :	Fructose
G- :	Pas d'inoculation de champignon mycorhizien à arbuscules
Gi :	Inoculation du *Glomus intraradices*
Gm :	Inoculation du *Glomus mosseae*
Gln :	Glutamine
Glu :	Glutamate
Gluc :	Glucose
Gly :	Glycine
h :	Heure
H :	Hydrogène
H+ :	Proton
HPLC :	Chromatographie en phase liquide de haute performance
H_2S :	Sulfure d'hydogène
HCN :	Cyanure d'hydrogène
HPO_4-H_2PO_4	Phosphate inorganique
Ile :	Isoleucine
INRA :	Institut national de recherche agronomique
IRBV :	Institut de recherche en biologie végétale
K :	Potassium
L ou l :	Litre
Leu :	Leucine
Lys :	Lysine
m :	Mètre
M :	Molaire
MS :	Spectrométrie de masse
min :	Minute
mg :	Milligramme
Mg :	Magnésium
MHB :	Mycorrhiza helper bacteria
mL ou ml:	Millilitre
mM :	Millimolaire
Mn :	Manganèse
MT :	Herbier Marie-Victorin, Montréal
MTT :	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide
N_2 :	Diazote
Na :	Sodium
Nb. :	Nombre
NH4+	Ammonium
No :	Numéro
OH :	Hydroxyde
P :	Phosphore

P :	Taux de probabilité
P+ :	Inoculation du *Phytophthora nicotianae*
P- :	Non-inoculation du *Phytophthora nicotianae*
PCR :	Polymerase Chain Reaction
PGPR :	Plant Growth Promoting Rhizobacteria
Phe :	Phénylalanine
Pi :	Phosphate inorganique
PR protein :	Pathogenesis related protein
Pr :	Professeur
Pro :	Proline
R^2 :	Coefficient de corrélation
rRNA :	ARN ribosomal
SE :	Erreur standard à la moyenne (Standard error of the mean)
sec :	Seconde
sem. :	Semaine
Ser :	Sérine
sp. :	Espèce
spp. :	Sous-espèce
Ri T-DNA :	Root inducing transformed deoxyribonicleic acid
RSI :	Résistance systémique induite (ISR)
RT-PCR :	PCR en temps réel (Real Time- PCR)
***Taq* polymerase :**	*Thermophilus aquaticus* DNA polymerase
TE :	Tris-EDTA, trishydroxyméthylaminométhane, acide éthylène-diamine-tétraacétique
Thr :	Thréonine
Trp :	Tryptophane
Tyr :	Tyrosine
TSA :	Tryptic Soy Agar
TSB :	Tryptic Soy Broth
var :	*Varietas*
v :	Volume
V :	Volt
Val :	Valine
w :	Poids (weight)
wk :	Week
Zn :	Zinc
% :	Pourcentage
°C :	Degrés Celcius
µ :	Taux de croissance spécifique
µL :	Microlitre

A mon grand-père,

Remerciements

Je tiens, dans un premier temps, à remercier le Pr. Marc St-Arnaud pour sa grande disponibilité, son soutien et les discussions autant stimulantes que constructives que nous avons échangées. Il a su me transmettre son immense intérêt pour l'étude des champignons mycorhiziens à arbuscules, la phytopathologie et le biocontrôle. Je tiens également à remercier mon second directeur, le Pr. Mario Jolicoeur, qui malgré une sollicitation moindre de ma part m'a transmis son soutien et son aide tout au long de ce projet.

Je voudrais également grandement remercier tous les étudiants stagiaires et employés que j'ai supervisés dans le laboratoire du Pr. M. St-Arnaud, sans qui ce projet n'aurait jamais pu être conduit à terme. Leur enthousiasme, leur disponibilité et surtout leur grande efficacité ont été fortement appréciés. Je voudrais particulièrement remercier Anne Keough qui n'est pas restée simplement une stagiaire mais une très bonne amie mais également François Perreault, Mylène Roitelle, Sébastien Loussouarn, Isabelle Barriault et Maude Beauregard tous également d'une très grande sympathie.

Vincent Manzanilla (étudiant à la maîtrise à l'Institut de recherche en biologie végétale, IRBV) a accepté de construire le programme nécessaire à l'arbre phylogénétique. Steve Hisiger (agent de recherche dans le laboratoire du Dr. M. Jolicoeur) a su trouver le temps d'effectuer les analyses HPLC. Les discussions à propos de statistiques avec Stéphane Daigle (statisticien à l'IRBV) étaient très instructives et je le remercie pour les analyses qu'il a lui-même effectuées pour ce

projet. Robert Mowatt (Société Plastroph, à Québec) a construit les microcosmes utilisés lors des expériences en sol.

Les discussions entretenues avec les Professeurs invités au laboratoire du Pr. M. St-Arnaud, Vladimir Vujanovic (Chaire AFIF en Microbiologie appliquée et en Biotechnologie, Département de Microbiologie appliquée et des Sciences des aliments, Collège d'Agriculture, Université de Saskatchewan, Saskatoon, SK) et Horst Vierheilig (Pr. Institut für Pflanzenschutz, Universität für Bodenkultur, Vienne) furent également très enrichissantes tout comme celles avec les autres étudiants des laboratoires des Pr. M. St-Arnaud et M. Jolicoeur, notamment celles avec Christine Martineau qui m'a donné une excellente formation en biologie moléculaire et avec qui j'ai beaucoup échangé.

Je tiens également à remercier l'ensemble des étudiants et professeurs de l'Institut de recherche en biologie végétale pour leur aide et l'ambiance exceptionnellement chaleureuse qui règne à l'institut. Les secrétaires Lucie Lemieux Sabourin, Lucie Campeau, Gina Calzuola et Lorène Lambert, l'agent comptable Thao Bubendorff et autre personnel de soutien Robert Cournoyer et Claude Lafleur sont aussi d'une aide inestimable permettant de mener à terme un tel projet que représente un doctorat.

Je veux également remercier mes amis autant de France que du Québec, Céd, Lolo, Julie, ma petite madeleine etc. pour leur grand soutien et les très bons moments que nous avons partagés. Et puis bien sûr ma famille qui malgré la distance et le manque est toujours derrière moi pour m'encourager.

Avant-propos

Je définirai ici ma contribution aux différents articles qui composent cette thèse. Elle est constituée de cinq articles : un de synthèse et quatre de recherche. L'article de synthèse (cf. chapitre III) a été accepté en vue d'une publication sous forme d'un chapitre de livre qui paraîtra à l'automne 2007. J'ai écrit la première version de la section : 'Arbuscular mycorrhizal fungi and their role in controlling soilborne plant-pathogens' ainsi que les versions subséquentes en tenant compte des commentaires des coauteurs et des éditeurs. J'ai aussi corrigé les autres sections de ce chapitre. Afin de le compléter, une synthèse bibliographique a été rédigée (cf. chapitre II). Les designs expérimentaux, les protocoles, les manipulations et les analyses statistiques des expériences nécessaires à la rédaction des articles de recherche ont été élaborés par moi-même, en accord avec mes directeurs de recherche. Ils ont été en totalité rédigés par moi-même et corrigés par mes directeurs. Ces articles seront prochainement soumis en vue de publication dans des revues scientifiques internationales.

François Perreault a effectué un certain nombre d'extractions d'ADN et de réactions de PCR nécessaires à la réalisation des gels de DGGE (cf. chapitre VI.). Anne Keough a contribué à la mise en place et effectué les biotests d'antagonisme des différents isolats bactériens isolés à partir de spores du *Glomus mosseae* envers différents agents pathogènes du sol (cf. chapitreVII). Ces étudiants étaient en stage d'initiation à la recherche du Baccalauréat en Sciences Biologiques de l'Université de Montréal et de l'Université du Québec à Montréal respectivement, sous ma supervision lors de la réalisation de ces travaux. Anne Keough a également occasionnellement aidé

aux manipulations nécessaires à la rédaction d'autres chapitres, tout comme d'autres étudiants d'été.

Steve Hisiger (agent de recherche dans le laboratoire du Pr. M. Jolicoeur) a effectué l'ensemble des analyses HPLC-MS du chapitre IV. Vincent Manzanilla (étudiant à la maîtrise dans le laboratoire du Pr. A. Bruneau, Institut de recherche en biologie végétale, Montréal) a mis en place le programme informatique nécessaire à la construction de l'arbre phylogénétique présenté dans le chapitre VII. Stéphane Daigle (statisticien, Institut de recherche en biologie végétale) a effectué les analyses statistiques des chapitres V et VI. Robert Mowatt (Plastroph, St-Etienne-de-Lauzon, QC) a construit les microcosmes décrits dans le chapitre V selon les plans que nous lui avons fournis.

Chapitre I

Introduction générale

L'agriculture intensive actuelle fait appel à l'emploi de pesticides en vue de contrebalancer les pertes de rendement en raison de l'induction de maladies par les agents pathogènes sur les espèces cultivées. Les agents de fumigation provoquent l'apparition de pollution des sols, les autres pesticides également polluants sont onéreux et induisent parfois la mise en place de résistances de la part des agents pathogènes. Ces produits sont par conséquent de plus en plus réglementés. Ainsi, l'utilisation de techniques telles la rotation culturale, la solarisation et la lutte biologique constituent des alternatives intéressantes qui permettraient de faire face à ces maladies de façon simple et respectueuse de l'environnement, dans une agriculture intégrée. La bioprotection (ou biocontrôle) est un moyen de lutte biologique qui consiste en la réduction des symptômes de la maladie et de la propagation de l'agent pathogène par l'application d'agents souvent antagonistes. Les champignons mycorhiziens à arbuscules (CMA) sont des champignons en symbiose avec les végétaux supérieurs qui entraînent fréquemment un meilleur développement de la plante hôte ainsi qu'une meilleure résistance aux stress abiotiques. La bioprotection par ces champignons a été démontrée dans de nombreux systèmes et s'explique par des mécanismes divers et parfois synergiques (St-Arnaud et Vujanovic, 2007; St-Arnaud et al., 1995a). La compréhension de ces mécanismes est indispensable afin d'optimiser l'utilisation des CMA en agriculture. Les répercussions sur la physiologie de la plante hôte ainsi que sur l'équilibre microbien du sol lors de l'épandage d'inoculas de CMA en champ ou de la gestion des populations mycorhiziennes naturellement présentes par des pratiques culturales appropriées doivent être compris et maîtrisés en vue d'utiliser efficacement ce moyen prometteur de lutte contre les maladies dont les végétaux sont victimes.

La tomate (*Solanum lycopersicum* L.) est une Solanacée de grand intérêt agronomique : elle est abondamment produite et consommée dans les pays industrialisés. Aussi elle est sujet à de nombreuses maladies causées par des champignons, des virus, des bactéries ou des nématodes (Blancard, 1988; Richard et Boivin, 1994). Une bioprotection, c'est-à-dire une diminution des effets négatifs sur la croissance de l'hôte, du nombre de points d'infection, du nombre de nécroses racinaires et du développement intraracinaire de l'agent pathogène, a été observée chez la tomate colonisée par le CMA *Glomus mosseae* et infectée par l'agent pathogène du sol le *Phytophthora nicotianae* Breda de Haan (Cordier et al., 1996; 1998; Pozo et al., 1996; 1998; 1999; 2002a). Une stimulation des mécanismes de résistance ainsi qu'une diminution du nombre de points d'infection ont été décrits dans ce système mais ils n'expliquent que partiellement la bioprotection observée.

Le *P. nicotianae* est un straménopile du sol provoquant des pourritures racinaires selon une faible spécificité d'hôte. Son principal mode de prolifération se fait par reproduction asexuée : lorsque les conditions environnementales sont optimales, des sporanges se forment et libèrent des zoospores. Celles-ci sont attirées par les exsudats des racines. Ces exsudats induisent alors l'enkystement et la germination des zoospores puis la formation des appressoria est provoquée par contact avec les tissus racinaires. L'agent pathogène pénètre et croît à l'intérieur de la racine hôte, met en place d'autres cycles de reproduction asexuée entraînant sa propagation rapide à l'intérieur des tissus hôtes et l'apparition des symptômes de la maladie. L'inhibition de ces étapes de préinfection limite fortement la propagation intraracinaire de l'agent pathogène permettant à la plante l'activation de systèmes de défense qui la protègent efficacement.

Une modification de l'exsudation racinaire (de façon qualitative et quantitative) par les CMA a été montrée (Azaizeh et al., 1995; Bansal et Mukerji 1994; Graham et al., 1981; Marschner et al., 1997; Sood, 2003). Notre première hypothèse est que ces modifications induiraient la mise en place de bioprotection chez la tomate infectée par le *P. nicotianae*. Elles provoqueraient un changement direct du comportement de l'agent pathogène, lors des étapes de préinfection, qui infecterait ainsi la plante sensible de façon moindre. De par leurs caractéristiques nutritionnelles et attractives, les exsudats racinaires permettent la mise en place de la rhizosphère : la zone du sol influencée par les racines et présentant une biomasse microbienne importante et spécifique. Il a été également démontré que les CMA modifient qualitativement et quantitativement la microflore de la rhizosphère, induisant l'apparition de la mycorhizosphère par des mécanismes encore mal connus. Notre seconde hypothèse est qu'un mécanisme important de la modification de la microflore de la rhizosphère par les CMA est la modification de la composition des exsudats racinaires induite par la colonisation. Cette microflore ainsi créée serait antagoniste des agents pathogènes et inhiberait sa prolifération dans le sol. Il a été montré que les CMA pouvaient servir de niche pour un certains nombre de microorganismes et constituent même parfois des hôtes obligatoires. Notre troisième hypothèse est que ces microorganismes seraient antagonistes des agents pathogènes du sol et seraient par conséquent impliqués dans le biocontrôle observé lors de l'inoculation de CMA.

Nos objectifs étaient de :

1. Montrer que les exsudats de racines mycorhiziennes extraits par culture monoxénique, *in vitro*, sont répulsifs ou moins attirants que les exsudats de racines non-mycorhiziennes vis-à-vis des zoospores du *P. nicotianae*.

2. Comparer la composition en sucres, acides aminés et acides organiques de ces exsudats afin d'identifier les molécules impliquées dans la modification du comportement des zoospores.

3. Vérifier que les exsudats de racines mycorhiziennes induisent l'apparition de bioprotection lorsque des plants de tomate infectés par le *P. nicotianae* sont cultivés en sol.

4. Montrer que les exsudats de racines mycorhiziennes modifient la microflore microbienne de la rhizosphère de tomate par utilisation de la technique de PCR-DGGE, à partir du gène 16S de l'ARN ribosomal.

5. Identifier les bactéries associées aux spores du *G. mosseae* par culture sur milieu standard et par la technique de PCR-DGGE, à partir du gène ribosomal 16S.

6. Examiner si ces bactéries isolées sont antagonistes d'un certain nombre d'agents pathogènes du sol (*P. nicotianae*, *Fusarium oxysporum* et *F. solani*).

Nous avons utilisé le cultivar *Cobra* de la tomate (*Solanum lycopersicum*), des racines transformées par l'*Agrobacterium rhizogenes* de ce cultivar (lignée 4C4) qui permettent une culture *in vitro* de ces racines dans des conditions axéniques ou monoxéniques étant disponibles dans notre laboratoire. La création du système *in vitro* compartimenté par St-Arnaud et al. (1996) nous a fortement inspirés pour la mise en place d'un système *in vitro* permettant de façon simple (dans un milieu liquide pauvre)

de collecter des exsudats de racines transformées afin de tester leurs effets sur l'attraction des zoospores de l'agent pathogène, sans l'intervention de microorganismes autres que le CMA testé. Le *G. intraradices* Schenck et Smith (DAOM 181 602), disponible *in vitro* (de façon axénique), a été utilisé pour l'expérience *in vitro*. Il a également servi pour l'expérience en sol tout comme le *G. mosseae* (Nicol. et Gerd.) Gerdemann et Trappe (BEG 12).

La technique de PCR-DGGE est une technique de biologie moléculaire utilisée depuis environ une dizaine d'années qui permet de façon simple et rapide d'étudier la communauté bactérienne ou fongique d'un milieu donné de façon quasi-complète (90% des espèces bactériennes peuvent être identifiées par amplification du gène 16S de l'ADN ribosomal) et présentant par conséquent de nombreux avantages par rapport aux techniques culturales conventionnelles qui sont laborieuses et donnent lieu à une identification inférieure à 10% de la communauté bactérienne. Cette technique s'avère être un outil intéressant en vue de la compréhension des interactions très complexes entre les CMA, les racines et les autres microorganismes du sol intervenant dans la mycorhizosphère.

Chapitre II

Synthèse bibliographique

II.1. Les champignons mycorhiziens à arbuscules

Les champignons mycorhiziens à arbuscules (CMA) établissent une symbiose endomycorhizienne de type mutualiste, c'est-à-dire bénéfique pour les deux partenaires, de manière non-spécifique (Sanders, 2002), avec environ 80% des plantes terrestres (Harley et Harley, 1987; Trappe, 1987; Wang et Qiu, 2006), depuis environ 400 millions d'années (Redecker et al., 2000; Remy et al., 1994; Simon et al., 1993; Taylor et al., 1995). Les CMA auraient permis aux végétaux supérieurs de conquérir les espaces terrestres grâce à leurs effets bénéfiques sur leur croissance et leur survie. Ils ont été décrits dans pratiquement tous les écosystèmes : les déserts, les forêts tropicales, les environnements aquatiques, dans les habitats arctiques et antarctiques (Strack et al., 2003). Ce n'est que récemment, grâce à des outils moléculaires, que depuis la classe des Zygomycètes, ils ont été placés dans le nouveau phylum *Glomeromycota*, divisé en quatre ordres : les *Diversisporales*, les *Paraglomerales*, les *Archaeosporales* et les *Glomerales*, ce dernier étant le plus représenté (Da Silva et al., 2006; Schüßler, 2002; Schüßler et al., 2001). Ils ne présentent pas de reproduction sexuée mais libèrent des spores asexuées multinucléées (Hijri et Sanders, 2005). Les hyphes formés suite à la germination de ces spores forment un appressorium lors d'un contact avec une racine hôte. Leur propagation, dans le cortex uniquement, s'effectue selon deux patrons possibles qui dépendent de l'espèce de CMA : *Paris* et *Arum*, ce dernier étant le plus représenté. Lors d'associations de type *Arum*, le champignon forme des hyphes inter et intra cellulaires, sans jamais franchir le plasmalemme. Mais parfois l'hyphe se divise de façon dichotomique invaginant la cellule hôte et formant une structure spécifique, appelée arbuscule, caractérisée par un contact étroit entre les membranes des deux

cellules symbiotiques et favorisant ainsi l'échange de nutriments entre les deux organismes. Dans le type *Paris*, l'hyphe très épais se propage principalement de façon intracellulaire, sans jamais franchir le plasmalemme, en formant des 'pelotes' de structure proche de celle des arbuscules (que nous nommerons structure arbusculée) (Smith et Read, 1997).

Des sucres marqués (glucose-C^{13} et fructose-C^{13}) et appliqués sur des racines mycorhiziennes ont été rapidement métabolisés par le champignon et convertis en trehalose, lipides et glycogène (Bago et al., 1999; Pfeffer et al., 1999). Par contre, si ces sucres étaient ajoutés au mycélium extraracinaire, aucun marquage des métabolites fongiques n'était observé, montrant ainsi que la fixation des composés carbonés par le champignon s'effectue à partir de la racine (Bago et al., 2000; Bécard et al., 2004; Lammers et al., 2001). Des enzymes de type H+-ATPases (possiblement impliquées dans le transport des composés carbonés) ont été identifiées au niveau des membranes fongiques des arbuscules et des hyphes intercellulaires (Gianinazzi-Pearson et al., 1991; Hause et Fester, 2005). Des transporteurs d'hexoses membranaires de racines seraient induits par la symbiose (Harrison, 1996).

Il a été montré que la nutrition phosphatée (Jakobsen et al., 1992; Pearson et Jakobson, 1993; Rhodes et Gerdemann, 1975), azotée (Bago et al., 1996; Cliquet et Stewart, 1993; Marschner, 1996) mais aussi du cuivre, du zinc, du potassium et du fer (Liu et al., 2000; Marschner, 1996; Smith et Read, 1997) des plantes terrestres étaient favorisées par la symbiose mycorhizienne. En effet, le réseau d'hyphes du champignon au niveau du sol explore un plus grand volume que les racines (George et al., 1995; Smith et Read, 1997). Des transporteurs de phosphate inorganique (Pi) au niveau de ces hyphes ont été identifiés (Harrison et van Buuren, 1995; Maldonado-Mendoza et al.,

2001). Une fois fixé, le Pi serait transformé en acides nucléiques, phospholipides ou en polyphosphates transportés vers le mycélium intraracinaire *via* des transporteurs qui demeurent inconnus (Ezawa et al., 2004; Karandashov et Bucher, 2005; Viereck et al., 2004). Le passage vers la racine se ferait sous forme de Pi par des transporteurs racinaires induits par la symbiose qui ont été identifiés au niveau des arbuscules et des structures arbusculées (Karandashov et Bucher, 2005; Ohtomo et Saito, 2005; Paszkowski, 2006). Les transporteurs racinaires directs du Pi (qui permettent directement sa fixation à partir du sol) seraient inactivés par la symbiose mycorhizienne, favorisant la fixation de ce minéral par l'intermédiaire des hyphes fongiques (Hause et Fester, 2005; Smith et al., 2003). Parmi l'absorption des autres minéraux que les CMA sont capables de fixer, celle de l'azote a été récemment élucidée. Le nitrate et le nitrite fixés par les hyphes extraracinaires seraient stockés sous forme d'arginine, elle-même transportée vers les structures intraracinaires du champignon. C'est sous forme de nitrate libéré à partir de la décomposition de cet acide aminé que l'azote serait transféré vers la racine (Govindarajulu et al., 2005; Jin et al., 2005).

En plus de présenter une fixation accrue des minéraux qui favorise leur croissance, les plantes en symbiose avec les CMA présentent d'autres caractéristiques. Leur tolérance aux métaux lourds ainsi que leur capacité à coloniser des sols contaminés sont augmentées notamment par le stockage au niveau des vésicules et des parois des hyphes de ces composés toxiques (Galli et al., 1994; Gildon et Tinker, 1981; Hildebrandt et al., 2007; Leyval et al., 1997). Les plantes colonisées par les CMA montrent également une plus grande tolérance à la sécheresse et au stress salin particulièrement grâce à l'augmentation de la biomasse et du réseau racinaires, à une

meilleure nutrition minérale et à l'augmentation du taux de photosynthèse et de la conductance des stomates (Allen et al., 1981; Augé et Duan, 1991; Levy et Krikun, 1980; Nelsen et Safir, 1982; Ruiz-Lozano et al., 1995; 1996; Schellenbaum et al., 1998). Le réseau d'hyphes au niveau du sol et la libération de la protéine glomaline par ces hyphes favorisent l'agrégation des sols (Wright et Upadhyaya, 1998; Wright et al., 1999). La synthèse hormonale (Fitze et al., 2005; Jentschel et al., 2007; Kaldorf et Ludwig-Müller, 2000a ; 2000b) mais aussi la quantité d'ADN totale et déméthylée ainsi que le niveau de ploïdie des cellules végétales sont augmentés suite à la mise en place de la symbiose, montrant un accroissement de leur métabolisme (Berta et al., 2000; Fusconi et al., 1997; Lingua et al., 2001). La symbiose avec les CMA entraîne aussi la réduction des symptômes de maladies, notamment ceux provoqués par les agents pathogènes du sol (champignons, protites, straménopiles et nématodes) mais aussi diminue les populations de larves d'insectes rhizophages (Gange, 2001; Gange et Nice, 1997) induisant ainsi un biocontrôle ou bioprotection (cf. chapitre III).

Les CMA présentent donc un certain nombre d'effets bénéfiques sur la croissance et la santé des plantes. Il a ainsi été montré que la biodiversité des CMA est un facteur prépondérant contribuant à la maintenance de la biodiversité des plantes et au fonctionnement des écosystèmes même si le nombre d'espèces décrites est très faible (environ 150) comparé au nombre d'espèces végétales (environ 200 000) (van der Heijden et al., 1998).

II.2. L'exsudation

De nombreuses revues bibliographiques détaillant les caractéristiques de l'exsudation et de la rhizosphère ont été publiées : Bais et al., 2006; Bertin et al., 2003; Bowen et Rovira, 1999; Curl et Truelove, 1986; Grayston et al., 1997; Hawes, 1990; Jones et al., 2004; Kuzyakov et Domanski, 2000; Lugtenberg et al., 2001; 2002; Lynch et Whipps, 1990; Nehl et al., 1997; Nguyen, 2003; Pinton et al., 2001; Uren, 2000; Uren et Reisenauer, 1988; Whipps, 1987; Whipps, 2001.

II.2.1. Définitions

La racine en croissance perd un certain nombre de composés libérés au niveau du sol sous forme de rhizodéposition constituée d'exsudats, de lysats et de cellules mortes. Environ 5 000 cellules de la coiffe sont relâchées dans le sol par jour par les racines de maïs. Des résultats similaires ont été obtenus avec d'autres plantes (Lynch et Whipps, 1990). Les exsudats peuvent être des composés solubles, du mucilage ou des gaz. Selon les différents auteurs, de 10 à 30% (Marschner, 1986), de 30 à 40% (Lee et Parkhurst, 1992) ou même de 40 à 46% (Helal et Sauerbeck, 1989) des composés synthétisés sont perdus sous forme d'exsudats chez les plantes annuelles. Il a été montré que les composés exsudés peuvent s'éloigner de 20 mm des racines de maïs en 25 jours (Lynch et Whipps, 1990).

Les microorganismes du sol exsudent également un certain nombre de composés. Les exsudats des CMA stimuleraient l'absorption des minéraux par la plante hôte (Tawaraya et al, 2006). Il a été montré que les exsudats mycorhiziens altéraient la croissance de certains microorganismes du sol (Filion et al., 1999) (cf. chapitre III).

II.2.2. Rôles de l'exsudation

L'exsudation racinaire permet l'augmentation de l'agrégation des sols limitant le lessivage des minéraux (Geelhoed et al., 1999; Grayston et al., 1997; Schilling et al., 1998), la détoxication des métaux lourds (Jones, 1998; Jones et al., 1996; 2004) et la réduction de la dessiccation de l'extrémité de la racine en limitant les frictions qui peuvent exister entre le sol et la racine lors de la croissance de cette dernière (Nehl et al., 1997). La fixation des minéraux (du Pi, mais aussi de l'azote et du fer) est favorisée grâce à la modification du pH du sol, de la libération d'enzymes, d'acides organiques et de ionophores qui augmentent leur solubilité (Pinton et al., 2001; Ryan et al., 2001; Schilling et al., 1998; Uren, 2000).

L'exsudation racinaire (notamment les sucres, les acides organiques et les acides aminés) constitue une source de carbone et d'énergie dans le sol (Knee et al., 2001) et l'activité microbienne y est élevée par rapport au sol distant permettant ainsi la mise en place de la rhizosphère. Celle-ci a été définie, en 1904 par Hiltner (1904), comme étant la portion du sol influencée par les racines et donc par les exsudats racinaires et caractérisée par une augmentation significative de l'activité microbienne mais aussi par des modifications qualitatives de la microflore (Bais et al., 2006; Baudoin et al., 2003; Campbell et al., 1997; Grayston et al., 1997; Griffiths et al., 1999; Jones et al., 2004; Nehl et al., 1997). Selon la nature des exsudats qu'elle libère dans le sol, la plante exerce une sélection différente sur les microorganismes qui se développent (Darrah, 1996; Grayston et al., 1997; Ibekwe et Kennedy, 1998; Shepherd et Davies, 1994). Les rhizobactéries peuvent avoir un effet bénéfique, neutre ou inhibiteur sur la croissance des plantes. Les 'Plant Growth Promoting Rhizobacteria' (PGPR) augmentent la

croissance et la morphologie des racines, la physiologie et le développement des plantes, la disponibilité des nutriments et leur absorption (Bowen et Rovira, 1999; Grayston et al., 1997; Nehl et al., 1997). Elles peuvent inhiber le développement des microorganismes pathogènes pour la plante (Azcón-Aguilar et Barea, 1996; Bowen et Rovira, 1999; Grayston et al., 1997; Nehl et al., 1997), stimuler la colonisation des racines par les CMA (Linderman, 1988) ou être fixatrices d'azote. Elles inhibent le développement des agents pathogènes par compétition pour les substrats carbonés, séquestration du Fe3+, lyse des cellules fongiques, induction de résistance systémique induite, relargage d'antibiotiques et augmentation de la disponibilité en Mn (Azcón-Aguilar et Barea, 1996; Bowen et Rovira, 1999; Larkin et Fravel, 1999; Nehl et al., 1997; Piga et al., 1997). Les Deleterious RhizoBacteria (DRB), quant à elles, inhibent le développement de la plante et sont souvent à l'origine de maladies. Elles peuvent produire des phytotoxines, inhiber le développement des CMA ou inhiber le développement des PGPR (Nehl et al., 1997).

Les métabolites secondaires libérés par les racines sont à l'origine de communication entre les végétaux ainsi qu'entre les végétaux et les microorganismes du sol (dictant l'apparition ou non de maladies ou de symbioses). Les plantes peuvent faire preuve d'allélopathie, c'est-à-dire libérer des phytotoxines affectant la physiologie, la croissance et la survie des plantes voisines sensibles ou des autotoxines responsables d'autoinhibition qui influencent de façon importante la biodiversité (Bais et al., 2004; 2006). La libération du facteur Nod nécessaire à la communication plante-*Rhizobium* et à la formation des nodules racinaires permettant la fixation d'azote atmosphérique par les légumineuses est induite par des flavonoïdes libérés par les racines (Djordjevic, 1987; Peters et Long, 1988; Peters et Verma, 1990). L'expression

des gènes de virulence par l'*Agrobacterium tumefasciens*, à l'origine de la galle du collet, est induite par des composés phénoliques (Hawes, 1990; Spencer et Towers, 1991; Stachel et al., 1985). La chématoxie, l'enkystement, la germination et ainsi la formation des appressoria de l'agent pathogène du sol *Phytophthora sojae* ont lieu en réponse à des flavonoïdes (la génistéine et la dadzéine) (Connolly et al., 1999; Deacon et Donaldson, 1993; Morris et al., 1995; Morris et Ward, 1992). Les espèces non hôtes des CMA, telles les *Brassicaceae*, libèreraient des isothyocyanates et des glucosinolates (Vierheilig, 2004; Vierheilig et Ocampo, 1990; Vierheilig et Piché, 2002; Vierheilig et al., 1990), les *Fabaceae* des isoflavonoïdes (Gagnon et al., 1995) et les *Chenopodiaceae* ne libèreraient pas les substances indispensables à la mise en place de mycorhization (Bécard et Fortin, 1988; Giovannetti et al., 1993). Le rôle des flavonoïdes dans la mise en place de la mycorhization est controversé (Bécard et al., 1995; Bécard et al., 2004; Buee et al., 2000) : certains (tels la quercétine) favoriseraient, d'autres inhiberaient la croissance des hyphes en direction des racines ainsi que la formation des appressoria (Balaji et al., 1995; Bel Rhlid et al., 1993; Chabot et al., 1992; Poulin et al., 1993). La balance entre les différents flavonoïdes exsudés dicterait l'intensité de mycorhization (Mandelbaum et Piché, 2000) et leur effet dépendrait du genre et même de l'espèce de CMA considérés (Scervino et al., 2005a) mais ne serait pas essentielle à la mise en place de mycorhization (Bécard et al., 2004; Requena et al., 2007). Récemment, le 'branching-factor', responsable de la ramification des hyphes de germination nécessaires à la mise en place de colonisation racinaire, a été identifié comme étant un strigolactone, 5-deoxy-strigol, à partir des exsudats racinaires de *Lotus japonicus* (Akiyama et al., 2005). En réponse à cette molécule, les CMA libéreraient un facteur Myc (équivalent du facteur Nod chez les *Rhizobium*) qui activerait l'expression

du gène *MtENOD11*, sans contact direct avec la racine, et serait ainsi à l'origine de la communication plante-CMA permettant la mise en place de la colonisation (Bécard et al., 2004; Harrison, 2005; Paszkowski, 2006).

II.2.3. Mécanismes et facteurs de variation de l'exsudation

L'exsudation se fait à proximité de la coiffe (McDougall, 1970; Rovira, 1973), de manière active ou passive, selon la taille des composés (Jones et Darrah, 1993b) et les espèces végétales.

Le taux d'exsudation racinaire est modifié par la concentration en phosphate dans le sol (Graham et al., 1981; Marschener, 1998; Ratnayake et al., 1978; Schilling et al., 1998), mais aussi des autres minéraux (Hodge et al., 1996; Liljeroth et al., 1994; Marschener, 1998; Schilling et al., 1998), par l'intensité lumineuse et la température (Graham et Leonard, 1982), le stade de développement, l'espèce et le cultivar de la plante (Johnson et al., 1982; Jones et Darrah, 1993b; Schilling et al., 1998; Shepherd et Davies, 1994; Whipps, 1987), la photopériode (Johnson et al., 1982), le type et le pH du sol (Grayston et al., 1997), la quantité d'oxygène et de dioxyde de carbone disponibles (Darrah, 1996; Hodge et al., 1998), la diversité des microorganismes présents au niveau de la rhizosphère (Meharg et Killham, 1995; Nehl et al., 1997) et le stress hydrique (Reid et Mexal, 1977).

La biomasse microbienne du sol augmente souvent l'exsudation des végétaux de manière quantitative, qualitative et spécifique (Garbaye, 1991; Nehl et al., 1997). Par exemple, les métabolites produits par *Pseudomonas aeruginosa* augmentent de douze fois l'exsudation de *Lolium perenne* (Meharg et Killham, 1995). Les microorganismes libèreraient des toxines ou créeraient un gradient d'exsudats au niveau de la rhizosphère

suite à leur minéralisation (Shepherd et Davies, 1994). Les CMA modifieraient l'exsudation de la plante hôte de façon quantitative et qualitative (cf. chapitre III).

II.2.4. Nature des exsudats

Les exsudats sont constitués de composés solubles dans l'eau (substances de faible poids moléculaire perdues passivement selon leur gradient de concentration), de sécrétions (de poids moléculaire plus grand et qui traversent la membrane des cellules selon un potentiel électrochimique ou chimique, comme les enzymes), de gaz (éthylène, CO_2, cyanure d'hydrogène, isothiol), de mucilage (principalement constitué de polysaccharides et d'acides galacturoniques de poids moléculaire élevé). En conditions non axéniques, le mucilage est une mixture d'origine végétale et microbienne appelée mucigel. La majorité des composés solubles dans l'eau sont des sucres, des acides organiques et des acides aminés puis des acides gras, des enzymes, des hormones et des vitamines (Bais et al., 2006; Bertin et al., 2003; Jones et al., 2004; Lynch et Whipps, 1990). Les sucres sont souvent les composés les plus exsudés (Grayston et al., 1997; Sood, 2003).

Le rôle des métabolites secondaires dans la communication entre les végétaux et les microorganismes a été montré à la section II.2.2. De très faibles concentrations (de l'ordre du nanogramme ou du picogramme), sont généralement suffisantes à l'induction des effets.

Le CO_2 est libéré par la respiration des racines, des CMA et des rhizobactéries. Il joue un grand rôle sur l'équilibre de la mycorhizosphère. Il stimule le développement des CMA (Balaji et al., 1995; Bécard et Piché, 1989; Chabot et al., 1992; Poulin et al.,

1993), à une concentration optimale de 2,0%, augmente l'exsudation des racines (Darrah, 1996; Hodge et al., 1998) et inhibe la croissance des bactéries aérobies du sol.

Les rhizobactéries libèrent, elles aussi, des produits à durée de vie plus ou moins longue qui peuvent rester au niveau du sol ou être absorbés par d'autres microorganismes, par la plante et ainsi modifier leur physiologie. Ces produits sont principalement des régulateurs de croissance (stimulateurs à faible concentration mais inhibiteurs à forte concentration), des acides organiques, du H_2S, HCN (phytotoxiques), des antibiotiques, des ionophores (qui augmentent la disponibilité du fer) ou des sidérophores (à effet inverse), des substances solubilisatrices de phosphore, des lectines, des agglutinines (permettant des agglutinations spécifiques de certains microorganismes aux racines), des polysaccharides (permettant une agglutination non spécifique et une agrégation du sol), des enzymes libres (permettant le contrôle des maladies pour un certain nombre d'entre elles) et des vitamines (Azcón-Aguilar et Barea, 1996; Bowen et Rovira, 1999; Linderman, 1988; Lynch et Whipps, 1990; Pinton et al., 2001; Sarwar et Kremer, 1995).

II.2.5. Mesure de l'exsudation

L'exsudation a d'abord été mesurée par simple dosage biochimique des sucres et des acides aminés rejetés par les racines (D'arcy, 1982; Graham et al., 1981; Lynch et Whipps, 1990; Schwab et al., 1983) puis l'ensemble des exsudats a été analysé par l'utilisation de techniques plus fines comme la chromatographie en phase liquide de haute performance, la chromatographie en phase gazeuse, la spectrométrie de masse et la résonnance magnétique nucléaire (Azaizeh et al., 1995; Lugtenberg et al., 1999).

Il est difficile de mesurer l'exsudation à cause de la présence de microorganismes dans le sol qui utilisent les exsudats (Grayston et al., 1997; Lynch et Whipps, 1990). De plus il existe une réabsorption des exsudats qui se fait tout le long du système racinaire : jusqu'à 90% des exsudats peuvent être réabsorbés chez le maïs (Jones et Darrah, 1993a; 1993b). Cette réabsorption se fait de manière active et est sélective de certains composés (Jones et Darrah, 1993a). Le fait d'enlever les exsudats au fur et à mesure de leur libération stimulerait leur libération (Jones et Darrah, 1993a).

II.3. Le *Phytophthora nicotianae*

II.3.1. Caractère généraux du *Phytophthora nicotianae*

Le *Phytophthora nicotianae* Breda de Haan, 1896, est un straménopile (*Oomycètes*, *Peronosporales*) responsable de pourritures racinanaires. Sa virulence a été montrée chez 72 genres et 40 familles d'angiospermes (Satour et Butler, 1967). Il est responsable d'une forte perte de rendement chez un grand nombre de plantes d'intérêt cultural tant chez les plantes herbacées telles la tomate, le tabac, le saintpaulia et la pomme de terre, que chez les arbres tels le citronnier, le pamplemoussier et le palmier. Cet agent pathogène du sol attaque en priorité les racines mais des nécroses, des fanes et une diminution de la biomasse peuvent aussi être observées aussi au niveau des parties aériennes chez les plantes sensibles (Satour et Butler, 1967). Les moyens de lutte actuels contre sa propagation prônent prioritairement la rotation culturale, la fumigation du sol et l'emploi de fongicides tels l'acide phosphorique, le fosetyl-Al et le métalaxyl (Erwin et Ribeiro, 1996).

Le genre *Phytophthora* possède un cycle de vie principalement diploïde (la méiose ayant lieu lors de la formation des cellules reproductrices) et est caractérisé par la présence d'une paroi majoritairement constituée de glucans (reliés par des liaisons β-1,3 et β-1,6) et de cellulose (et non de chitine, contrairement aux champignons supérieurs). Son principal mode de prolifération est également particulier et se fait par la différentiation d'un grand nombre de spores mobiles (nommées zoospores) formées par reproduction asexuée (Erwin et Ribeiro, 1996; Erwin et al., 1983). La diminution de l'attraction des zoospores par les racines, de la quantité de zoospores qui s'enkystent puis germent et forment des appressoria à la surface des racines permet à la plante

d'activer des systèmes de défense efficaces et ainsi de limiter l'infection. Par contre, si ces étapes de préinfection ne sont pas perturbées, de nombreux cycles de reproduction asexuée se mettent en place et l'agent pathogène se propage et induit l'apparition de symptômes rapidement (Erwin et Ribeiro, 1996; Erwin et al., 1983).

II.3.2. Etudes concernant la bioprotection des champignons mycorhiziens à arbuscules sur le *Phytophthora nicotianae*, chez la tomate

Un certain nombre d'études récentes montrent qu'une précolonisation par le *Glomus mosseae* (Nicol. et Gerd.) Gerdemann et Trappe provoque une bioprotection chez la tomate infectée par le *P. nicotianae* (Cordier et al., 1996; Pozo et al., 2002a; Trotta et al., 1996; Vigo et al., 2000). Dans ce contexte, une réduction du nombre de nécroses, une baisse du développement du microorganisme pathogène, ainsi qu'une diminution de l'inhibition de la croissance de l'hôte ont été observées. Cette bioprotection se ferait de façon systémique car elle a été observée (dans un système compartimenté de type split-root) au niveau de racines d'un compartiment non-colonisé et d'un compartiment directement inoculé par le CMA de manière analogue (Pozo et al., 2002a). Les mécanismes proposés n'élucident que partiellement cette bioprotection. L'augmentation de la nutrition de l'hôte (notamment en phosphore) qui permettrait à la plante de compenser les dommages causés par l'infection par l'agent pathogène ne serait pas impliquée (Trotta et al., 1996). Mais la stimulation des mécanismes de défense de la plante hôte suite à la colonisation a été montrée. L'augmentation de la synthèse de β-1,3-glucanase (synthétisée de façon constitutive) mais aussi des isoformes spécifiques de chitinase et de β-1,3-glucanase ont été détectées dans des racines colonisées par le *G. mosseae* (Pozo et al., 1996; 1998; 1999). Cordier et al.

(1998) ont identifié dans des racines infectées par le *P. nicotianae* des modifications pariétales locales telle l'accumulation de callose autour des cellules corticales contenant des arbuscules mais aussi l'apparition d'épaississements pariétaux contenant des pectines et de protéines de type PR-1 caractéristiques de la mise en place de résistance systémique induite (RSI) au niveau de cellules racinaires non colonisées de plants colonisés par le *G. mosseae*. Le dénombrement des points d'infection induits par l'agent pathogène a permis d'établir que ceux-ci étaient réduits suite à la colonisation par le *G. mosseae*. La prolifération du *P. nicotianae* serait réduite dans la mycorhizosphère, avant que celui-ci n'envahisse la racine et la modification de l'équilibre de la rhizosphère pourrait être impliquée dans ce système (une baisse du nombre de nécroses étant observée en des points distants des zones racinaires colonisées par le champignon endomycorhizien) (Cordier et al., 1996; 1998; Pozo et al., 2002a; Vigo et al., 2000).

II.3.3. La reproduction asexuée chez les Oomycètes

Les caractéristiques ainsi que la description des facteurs qui influencent la reproduction asexuée des Oomycètes ont été revues par plusieurs auteurs (Carlile, 1983; Deacon et Donaldson, 1993; Erwin et Ribeiro, 1996; Ribeiro, 1983; van West et al., 2003).

II.3.3.1. Formation et chémotaxie des zoospores

Les conditions qui induisent la mise en place de la reproduction asexuée chez les Oomycètes sont une température élevée (entre 20 et 25°C) et une forte humidité (une pluie importante) qui entraînent l'appauvrissement du milieu. Des sporanges se forment à l'extrémité des hyphes et différencient des zoospores qui, libérées dans le milieu

liquide, nagent en direction des racines (Royle et Hickman, 1964a; 1964b). Elles sont attirées préférentiellement par la zone sous-apicale de la racine (où la majorité des exsudats sont libérés) (Ho et Hickman, 1967b). Les zoospores sont de forme ovoïde, ne sont pas recouvertes d'une paroi et possèdent un noyau excentré ainsi qu'un grand nombre de vésicules périphériques. Elles sont constituées de deux flagelles leur permettant un mouvement en ellipse et sur elles-mêmes. Leur temps de survie est fortement influencé par les conditions environnementales (Ho et Hickman, 1967a).

Une attraction par les exsudats racinaires a été montrée *in vitro* (Deacon et Donaldson, 1993; Hickman, 1970; Ho et Hickman, 1967a; 1967b). Les différents comportements adoptés par une zoospore à proximité d'une substance (chémotaxie) ont été décrits (Royle et Hickman, 1964a; 1964b), permettant de graduer qualitativement l'attraction ou non d'une zoospore vis-à vis d'une substance. Des mesures quantitatives ont permis également de montrer une attraction différentielle en fonction du pH (Morris et al., 1995) et des substances testées auxquelles les zoospores sont exposées : elles sont attirées par différentes substances hydrophobes [tel l'éthanol, (Allen et Newhook, 1973)], hydrophiles [les sucres mais aussi les acides aminés tels l'acide glutamique et l'acide aspartique (Donaldson et Deacon, 1993a; 1993b; Halsall, 1975; Hickman, 1970; Jones et al., 1991; Khew et Zentmyer, 1973; Leaño et al., 1998)] ou volatiles (Tableau I). Toutefois, la chémotaxie observée dépend des conditions expérimentales (Deacon et Donaldson, 1993) et de l'espèce étudiée : plus une espèce est spécifique d'un hôte, plus ses zoospores sont attirées par des molécules précises. Par exemple, le *Phytophthora sojae* qui n'est virulent que sur le soja libère des zoospores attirées spécifiquement par la dadzéine et la génistéine (des flavonoïdes)(Connolly et al., 1999; Deacon, 1996; Morris et Ward, 1992). Il a été montré que les zoospores du *P. nicotianae* étaient

Tableau I. Facteurs capables de provoquer la mise en place des étapes de pré-infection racinaires par les Oomycètes, suite à la libération de zoospores par reproduction asexuée

Facteurs impliqués	**Chémotaxie**	***Enkystement***	**Germination**	**Tropisme du tube germinatif**	**Formation des appressoria**
					
Molécules solubles	Acides aminés (ac. aspartique, ac. glutamique), Sucres Aldéhydes Isoflavones	Acides aminés (ac. aspartique, ac. glutamique) Isoflavones	Acides aminés (ac. aspartique, ac. glutamique) Sucres	Acides aminés (méthionine, phénylalanine) Aldéhydes Isoflavones	Nutriments, Substances hydrophiles
Molécules volatiles	Alcools Ethylène Acides gras volatiles			Alcools	
Molécules présentes à la surface de la racine	Isoflavones	Polysac-charides : Fucose Cellulose Chitine Polyuronates Isoflavones	Isoflavones		Glycoprotéines
Autres facteurs	pH Champ Electrique Anticorps	Anticorps		Champ électrique	Anticorps
Facteurs physiques	Champ magnétique	Température Choc osmotique Contact avec une surface		Aération	Contact avec une surface Chocs thermiques et osmotiques
Facteurs particuliers	Autoaggrégation		Possiblement programmée		
Rôle du calcium	Gouverne le patron de mobilité	Indispensable à l'adhésion	Doit être libéré au niveau du cytoplasme	Nécessaire à la croissance de l'hyphe	Doit être libéré au niveau du cytoplasme

attirées par des sucres (le sucrose, le dextrose, le fructose, le rhamnose et le maltose)(Dukes et Apple, 1961) mais également par des acides aminés (l'asparagine et la glutamine), dans de plus faibles proportions (Halsall, 1975). La chémotaxie se ferait par activation de récepteurs : les zoospores ne se nourrissent pas (Deacon et Donaldson, 1993) et leur agrégation est provoquée par des anticorps (Addepalli et Fujita, 2001; Estrada-Garcia et al., 1990; Hardham et al., 1994). Néanmoins, elles sont également attirées par l'anode dans un champ électrique (Khew et Zentmyer, 1973; Morris et al., 1995; van West et al., 2003). Aussi, elles sont capables d'autoagrégation (Reid et al., 1995).

II.3.3.2. Enkystement des zoospores

Les zoospores qui s'enkystent adoptent une forme arrondie, leur noyau migre vers le centre, les flagelles se désagrègent, les vésicules sont exocytées et une paroi périphérique est synthétisée (Ho et Hickman, 1967a). La spore enkystée s'accroche alors à un support (une racine ou un support inerte) de façon précise et orientée vers les substances attractives. Avant de s'enkyster, la zoospore libère une grande quantité de calcium (Connolly et al., 1999). L'enkystement serait provoqué par la reconnaissance de substances de surface racinaire ou du mucilage de l'hôte (la cellulose, le glucan, des polyuronanes tels l'acide uronique, le fucose et d'autres polysaccharides)(Donaldson et Deacon, 1993a; 1993b; Estrada-Garcia et al., 1990; Jones et al., 1991)] mais aussi par des substances chémoattractives telles des acides aminés (l'acide glutamique et l'acide aspartique, notamment)(Jones et al., 1991) ou des isoflavones qui seraient libérés dans les exsudats (Tableau I). Tout comme pour la chémotaxie, des récepteurs seraient impliqués : ils se lieraient à des substances plus ou moins particulières selon la spécificité de l'interaction hôte-agent pathogène (Donaldson et Deacon, 1993a).

Toutefois des températures et des pH extrêmes, des chocs mécaniques, thermiques et osmotiques et des lectines peuvent provoquer l'enkystement des zoospores de manière non-spécifique permettant ainsi une survie de l'organisme dans des conditions non-favorables (Deacon et Donaldson, 1993).

II.3.3.3. Germination des spores

La spore enkystée s'est orientée lors de l'enkystement de façon à ce que la germination se fasse en direction de la racine ou des substances ayant provoqué l'enkystement. Le point de germination est fixe : à proximité du point où étaient ancrés les flagelles. La germination serait induite par une forte concentration cytoplasmique en calcium qui proviendrait soit des réserves de la zoospore enkystée, soit de l'extérieur (Connolly et al., 1999). Deux théories s'opposent quant aux mécanismes de mise en place de la germination : certains auteurs pensent qu'une cascade d'événements est stimulée par l'enkystement de la zoospore, mettant en jeu des flux de calcium. Toutefois, un certain nombre de molécules tels des acides aminés (notamment l'acide glutamique et l'acide aspartique), des sucres (tel le glucose), le gellan-gum et des extraits peptoniques de racines stimulent ou provoquent la germination (Deacon et Saxena, 1998; Donaldson et Deacon, 1993a; 1993b; Jones et al., 1991) (Tableau I). Ils se lieraient à des récepteurs présents à la surface de la paroi de la spore et provoqueraient une augmentation du taux de calcium intracytoplasmique (Deacon et Donaldson, 1993).

Dans des conditions non-favorables (lorsque les réserves de la zoospore sont faibles, en présence d'une faible concentration en nutriments, particulièrement en calcium et/ou en absence de reconnaissance de molécules de surface de la racine), la spore ne germe pas mais libère des zoospores secondaires qui constitueraient un moyen

d'envahir des racines fortement distancées de la zone de libération des zoospores primaires (Connolly et al., 1999; Hill et al., 1998; Jones et al., 1991; Reid et al., 1995; Von Broembsen et Deacon, 1996; Warburton et Deacon, 1998; Xu et Morris, 1998).

II.3.3.4. Tropisme de l'hyphe germinatif

L'extrémité de l'hyphe de germination contiendrait des récepteurs sensibles à certaines molécules telles des acides aminés (particulièrement la méthionine et la phénylalanine), des aldéhydes et des alcools (Jones et al., 1991)(Tableau I). Cependant il serait fort possible que le tropisme de l'hyphe germinatif en direction de la racine se fasse selon un gradient de nutriments sans faire appel à des récepteurs (Deacon et Donaldson, 1993).

II.3.3.5. Formation des appressoria

En contact avec une racine, les appressoria appliquent une pression mécanique sur la paroi racinaire et induisent la formation d'hyphes secondaires dont la croissance permet la propagation de l'agent pathogène à l'intérieur des tissus de l'hôte. La formation des appressoria est un phénomène encore mal compris et principalement décrit chez les *Phytophthora* (le *Phytophthora infestans* et le *Phytophthora megasperma*) dont les zoospores infectent l'hôte en empruntant ses parties aériennes. Dans ces cas, elle peut être provoquée par des chocs thermique ou osmotique ou par simple contact avec une surface solide et est fortement influencée par la topographie, l'hydrophobicité et la disponibilité en nutriments (Bircher et Hohl, 1997a; 1997b). Elle fait appel à des glycoprotéines de surface (situées à l'extrémité du tube germinatif) qui, une fois activées, entraînent la mise en place de réactions en chaîne nécessitant l'entrée de calcium à l'intérieur du cytoplasme de la cellule (Bircher et Hohl, 1999).

L'apparition des premiers appressoria a lieu, en général, une heure après l'enkystement des zoospores pour la plupart des espèces de *Phytophthora*.

Chapitre III

Interactions between arbuscular mycorrhiza and soil microorganisms

Ce chapitre a été accepté afin de constituer un chapitre de livre, selon la référence suivante :

Laëtitia Lioussanne[1], Marie-Soleil Beauregard[1], Chantal Hamel[2], Mario Jolicoeur[3] & Marc St-Arnaud[1*]. Sous presse. Interactions between arbuscular mycorrhizal fungi and soil microorganisms. Dans *Advances in Mycorhizal Biotechnology:A Canadian Perspective* (ed. Khasa D., Piché Y. et Coughlan A.), Chapter 12. NRC Press.

[1] Institut de recherche en biologie végétale, Jardin botanique de Montréal, 4001, Sherbrooke Est, Montréal, QC, Canada, H1X 2B2

[2] Environmental Health/Water and Air qualiy, Agriculture and Agri-Food Canada, 1 Airport Road, Box 1030, Swift Current, SK, Canada, S9H 2X3

[3] Bio-P2 Research Unit, Department of Chemical Engineering, École Polytechnique de Montréal, P.O. Box 6079, Centre-ville Station, Montréal, QC, Canada, H3C 3A7

Introduction

Bacteria, fungi, algae, nematodes and protozoa are the principal components of the soil microflora and fauna. Most of these depend on organic matter as a source of C and generally proliferate in the top 10 to 15 cm of the soil profile, where plant roots and organic residues are most abundant. Soil microbial communities are essential for nutrient cycling, plant growth and, as a result, life on Earth. The extent to which these communities interact amongst themselves and with plant roots is thus of great importance. These interactions are complex and may fall anywhere along the continuum that ranges from wholly mutualistic to wholly pathogenic.

Soil microbial communities are species rich; however, they show an apparently high degree of functional redundancy, and the notion of functional groups is frequently employed when treating this subject. Arbuscular mycorrhizal (AM) fungi form one such group and have been coevolving symbiotically with plants for more than 400 M yr. (Remy et al., 1994). These fungi act as root extensions, increasing the volume of soil influenced and exploited by plants (Figs. 1 and 2). As a major interface between plant roots and the soil, they play a pivotal role in nutrient cycling (Dodd, 2000; Hodge, 2000) and ecosystem productivity (van der Heijden et al., 1998), and influence the outcome of interactions between plants (Klironomos, 2002). They are therefore ecologically important for plants by improving the physical quality of soil (Jastrow et al., 1998; Six et al., 2004), supplying mineral nutrients and water to host plants (Subramanian and Charest, 1998; Smith et al., 2001), and reducing the impact of root pathogens (St-Arnaud and Vujanovic, 2007). Therefore, mycorrhizal fungi must be considered when developing management strategies for sustainable soil-plant systems.

Moreover, it is important to understand how soil microbial communities influence, and are influenced, by AM fungi. This chapter provides a summary of some of the recent advanced in our understanding of microbial interactions in the mycorrhizosphere, and particular emphasis is placed on their role in nutrient cycling and plant health.

Arbuscular mycorrhizal fungi and general soil microbial diversity

The composition of the microbial community varies with the nature of the soil environment; however, its biomass is usually positively correlated with soil organic matter (SOM) content (Witter and Kanal, 1998; Manjaiah et al., 2000; Bohme et al., 2005) or C availability (Campbell et al., 1999). Most of the 10^6 to 10^9 microbial units typically present in a single gram of soil are bacteria and fungi. The latter normally constitute the largest component of the soil microbial biomass (Domsch et al., 1980; Atlas and Bartha, 1997; Brodie et al., 2003) and their hyphae may form extensive mycelial networks (Brodie et al., 2003) within the soil matrix. Of these fungi, those forming arbuscular mycorrhizal associations are typically highly abundant, and may account for approximately 25% of the microbial biomass (Hamel et al., 1991; Olsson et al., 1999; Hamel, 2007) and up to 80% of the fungal biomass (Kabir et al., 1997; Bååth et al., 2004) in certain agricultural soils.

The distribution of the major mycorrhizal associations in ecosystems generally follows altitudinal and/or latitudinal gradients (Read, 1991; Smith and Read, 1997), with AM being most abundant in temperate deciduous forests, grasslands, agricultural systems and tropical forests, where P availability typically limits productivity. Arbuscular mycorrhizal fungi are, therefore, an important component of Canada's agricultural and forest ecosystems (Dalpé, 2003).

Figure 1. Root-organ culture of *Glomus intraradices* on transformed carrot roots. The arbuscular mycorrhizal fungi mycelium grows in the soil at distance from the host roots, acting as root extensions and enlarging the volume of soil exploited by plants, as well as enhancing the influence of plants on the soil environment.

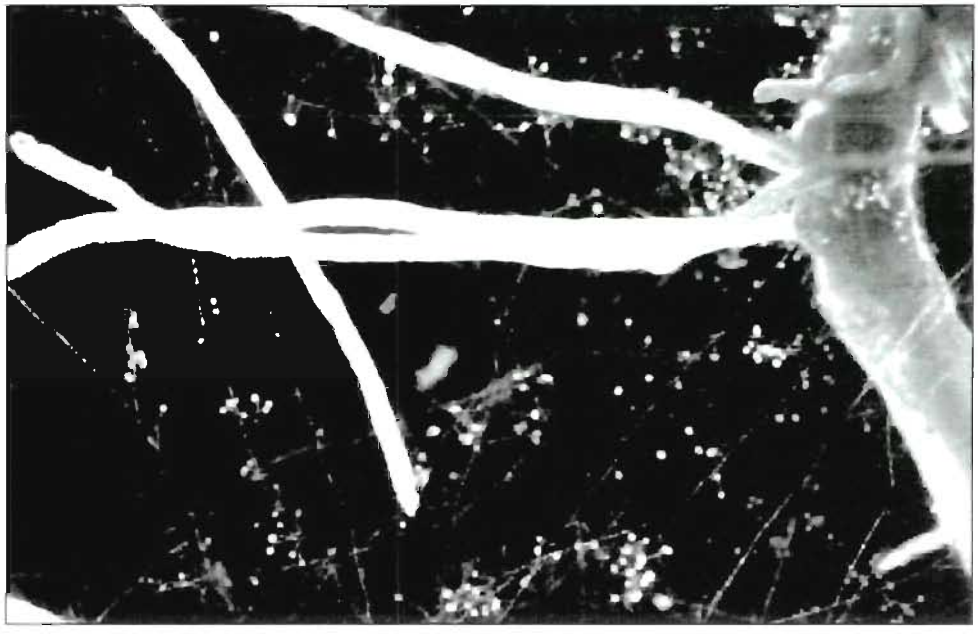


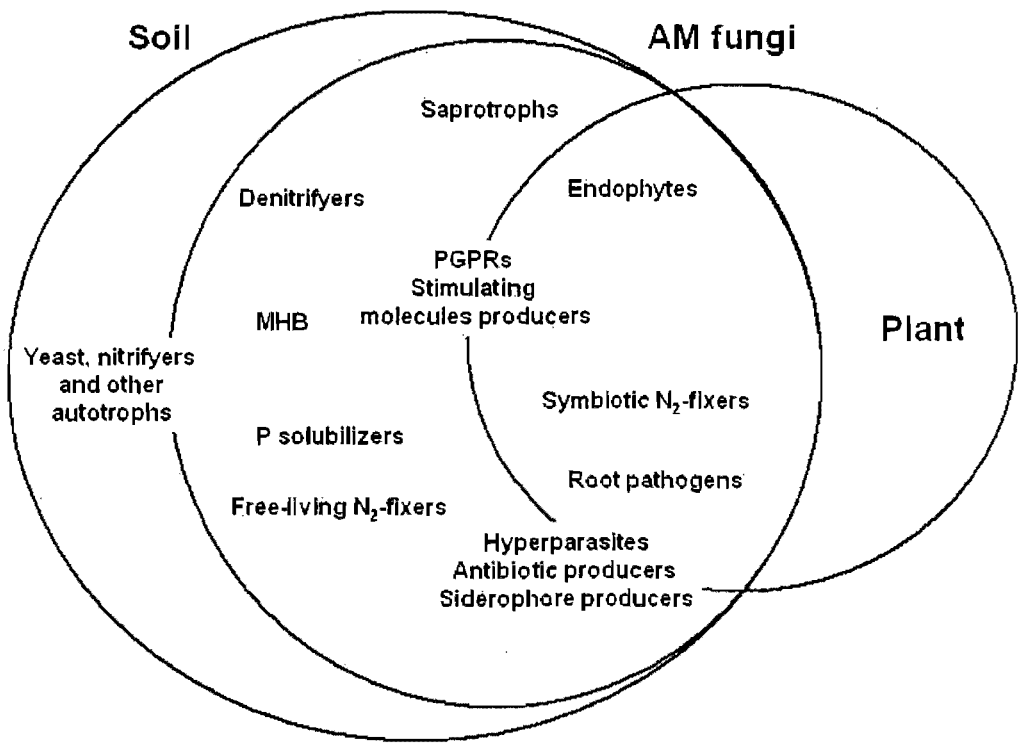
Figure 1. Root-organ culture of *Glomus intraradices* on transformed carrot roots. The arbuscular mycorrhizal fungi mycelium grows in the soil at distance from the host roots, acting as root extensions and enlarging the volume of soil exploited by plants, as well as enhancing the influence of plants on the soil environment.

Biological interactions in soil

A soil microorganism may be affected by the presence of plant roots, other microorganisms and diverse environmental conditions. As much as 10-40% of the C fixed by plants through photosynthesis is released in the soil in forms readily available for soil microorganisms, stimulating microbial activity (Bertin et al., 2003; Bowen and Rovira, 1999; Uren, 2000). Consequently, rhizodeposition is an important factor coupling plant nutrition and microbial processes. Much of the soil microbial activity is thus spatially organized around the roots, in this particularly active zone of soil called the rhizosphere. Active release of organic materials by roots occurs mostly at the root tips where outer root cap cells secrete mucilage, such as polysaccharides and proteins (Sievers and Braun, 1996). The effect of plants on soil microorganisms depends on the plant species in question and its growth phase (Söderberg et al., 2002; Wamberg et al., 2003).

The potential of plants to influence soil microorganisms is highlighted by the occurrence of different microbial populations in the rhizosphere of different plant species in a given community (Ibekwe and Kennedy, 1998; Marschner et al., 2001), or the presence of similar microbial communities in the rhizospere of a given plant species grown in different soils (Grayston et al., 1998; Miethling et al., 2000). Different AM fungal communities may occur in different plant communities (Zhang et al., 2004), on different plant species (Saito et al., 2004) and even on different parts of the same root (Scheublin et al., 2004). While the reason for these differences is poorly understood, differences in the population of AM fungi with soil depth (Oehl et al., 2005) are likely a result of root distribution.

Figure 2. Diagrammatic representation of the physico-chemical environment in which interactions between AM fungi and soil microorganisms are taking place. The distance between a microbial group and the plant, AM fungi or soil spheres indicates the presumed influence level of these spheres on the microbial group. AM fungi enhance plant photosynthesis and C export below ground since AM fungi are C-sinks on plants. AM hyphae proliferation throught the soil rooting zone modify non-humic C distribution in soil. This C is a important microbial food in soil, where microbial biomass is normally C-limited. The production of inhibitory molecules by AM fungi was never reported, but AM fungi may compete for soil resources with other soil organisms. Autotrophs are not stimulated by AM fungi as C source, but may be influenced by them indirectly, through their impact on soil nutrient levels and interaction with other soil organisms. Interactions between AM fungi and members of the main functional groups of microorganisms illustrated here, can be positive, negative or neutral, and likely result from the complex dynamic equilibrium between living organisms and environmental conditions that exists in soil.



Mycorrhizal fungi effectively extend the influence of plants beyond the rhizosphere, and interact directly and indirectly with other soil microorganisms. These interactions may lead to positive or negative changes in the populations of the different components of the soil microbial community and lead to the formation of the so-called mycorrhizosphere (Meyer and Linderman, 1986a, 1986b; Paulitz and Linderman, 1989; Calvet et al., 1992; St-Arnaud et al., 1995b; Rousseau et al., 1996; Filion et al., 1999; Vigo et al., 2000; Elsen et al., 2001; Talavera et al., 2001; Gryndler et al., 2002; St-Arnaud and Elsen, 2005).

Microbial species differ in their ability to metabolize or access different nutrient sources (Baudoin et al., 2003). Formation of AM induces changes in root exudation patterns, modifying the plant's impact on the soil biota. Furthermore, the extraradical hyphae become an important source of exudates (Linderman, 1992; Jakobsen et al., 2002), which comprised C of plant origin (Nakano et al., 1999; Johnson et al., 2002). This is supported by the changes both in the total microbial population and ratio between specific microbial taxa found in the hyphosphere of mycorrhizal root systems (the root-free zone of soil influenced by mycorrhizal structures) compared to the rhizosphere of non AM plants (Posta et al., 1994; Andrade et al., 1998; Ravnskov et al., 1999; Marschner et al., 2001; Jeffries et al., 2003). Direct interactions between fungal hypha and bacteria are diverse and involve mutualistic exudate-consuming bacteria that are associated with fungal surfaces as well as endosymbiotic and mycophagous species (Boer et al., 2005). The positive selection for mycorrhizal fungal-specific bacteria most likely occurs under a given plant (Linderman and Paulitz, 1990; Olsson et al., 1996a).

Several studies have shown qualitative, quantitative and spatial shifts in bacterial communities due to mycorrhizal associations (Meyer and Linderman, 1986b;

Linderman and Paulitz, 1990; Posta et al., 1994; Andrade et al., 1997). Furthermore, there are numerous reports of AM fungi influencing bacterial growth rate (Christensen and Jakobsen, 1993; Marschner and Crowley, 1996a, 1996b; Marschner et al., 1997). For example, growth of *Pseudomonas chlororaphis* was positively correlated with the concentration of unidentified components within the AM fungus hyphosphere. By contrast, growth of *Clavibacter michiganensis* was not affected (Filion et al., 1999).

Arbuscular mycorrhizal fungi may also affect soilborne fungi. The outcome of interactions with saprophytic species (Larsen et al., 1998; Olsson et al., 1998; Green et al., 1999) is difficult to predict. For example, negative, neutral and positive effects of AM fungi on the population density of species of *Trichoderma* have been observed (Fracchia et al., 1998; Godeas et al., 1999; Green et al., 1999). Furthermore, recent results revealed that *Trichoderma pseudokoningii* influenced AM fungal development and function, thus potentially modifying the impact of AM fungi on soil and plants; however, the effect varied with the saprophytic fungus strain used (Martinez et al., 2004). A number of studies also indicate that saprotrophic fungi influence pre-symbiotic AM fungi: positive and negative spore germination and germ-tube growth responses to volatiles and soluble exudates from saprotrophic fungi have been observed (Fracchia et al., 1998; McAllister et al., 1994, 1995). Furthermore, saprotrophic fungi may affect the functioning of AM fungi within host roots (McAllister et al., 1994, 1995; Fracchia et al., 1998; Garcia Romera et al., 1998; Martinez et al., 2004).. However, the nature of these interactions is still unclear.

Yeasts may also influence AM fungi. For example, *Rhodotorula mucilaginosa*, *Cryptococcus laurentii* and *Saccharomyces kunashirensis*, or their soluble and volatile exudates, were shown to stimulate *G. mosseae* spore germination, hyphal growth and

the development of the intraradical phase (Sampedro et al., 2004). Studies using *Saccharomyces cerevisiae* gave similar results (Larsen and Jakobsen, 1996). Furthermore, when *Yarowia lipolytica* was inoculated into a desertified soil, a 187 % increase in AM fungal biodiversity was recorded (Medina et al., 2004a, 2004b).

Different AM fungal species may also interact between each other. In a microcosm experiment, plant productivity increased in the presence of several AM fungal species (Klironomos et al., 1998). However, inoculation of strawberry cultivars with a mixture of *Glomus intraradices*, *G. mosseae* and *G. etunicatum* did not increase yield and sometimes reduced mycorrhizal development, when compared to inoculation with *G. intraradices* alone (Stewart et al., 2005).

Various microorganisms, including isolates of species of *Azotobacter*, *Azospirillum*, *Bacillus*, *Clostridium* and *Pseudomonas*, have been shown to improve plant growth by producing growth-enhancing substances and suppressing root pathogens (Glick, 1995; Jeffries and Dodd, 1996; Vazquez et al., 2000; Siddiqui and Shaukat, 2002; Vessey, 2003; Gamalero et al., 2004). Collectively, these organisms are referred to as plant growth promoting rhizomicroorganisms (PGPRs). Certain PGPRs also influence AM fungi. For example, it was shown that the inoculation of *Ficus benjamina* with two PGPRs (*Bacillus coagulans* and *T. harzianum*) and an AM fungus (*G. mosseae*) not only positively affected plant biomass, but also enhanced root colonisation by the AM fungus. This suggests a synergistic interaction between the three microorganisms (Srinath et al., 2003). By contrast, inoculation of strawberry plants with various combinations of *G. mosseae* and four PGPRs, in an attempt to reduce the levels of infection caused by *Phytophthora*, gave varying results that ranged from inhibition of the pathogen, to its stimulation (Vestberg et al., 2004). Interactions

between soilborne plant pathogenic microorganisms and AM fungi are treated in greater depth below.

Little is currently known about the ecology of AM fungi under natural conditions (Hodge, 2000). However, the above examples help to illustrate that the outcome of interactions between AM fungi and diverse soil microorganisms may be highly variable and difficult to predict. This suggests that the environmental conditions under which they occur may be important, and that a better understanding of their ecology might be obtained by taking soil type into account. Different soil types offer different conditions for the soil biota. Rivera and Fernández (2003) found good relationships between AM fungal species and soil types. Crop yield increases were obtained mainly when AM fungal species were inoculated in their corresponding soil type. As soils are more easily described than the soil microbial communities themselves, soil classification may be an important tool for the successful application of AM fungi to different agricultural systems.

Arbuscular mycorrhiza in nutrient mobilization

AM fungi enhance plant uptake of poorly mobile nutrients (Barea et al., 2005) and those present in low concentrations by increasing the volume of soil exploited and mobilizing the nutrients more efficiently than roots. By removing nutrients from the soil solution, AM fungi disrupt the equilibrium that exists between the amount of a given nutrient present in solution and the amount on the solid phase, which triggers further release from the latter. Furthermore, AM fungi are able to influence the mobilization of soil nutrients via the transport of C into the soil matrix. This effect is indirect and occurs as a result of the stimulation of soil microorganisms involved in nutrient cycling. The subsequent biological activity causes the breakdown and mineralization of SOM and solubilization of mineral materials (Paul and Clark, 1996).

Most soil microorganisms are involved in the mineralization of soil organic P and are considered to contribute significantly to the total soil phosphatase activity (Richardson, 1994). To maintain their metabolism, soil microorganisms either use C released from plant roots and/or mycorrhizal fungi (Sievers and Braun, 1996; Bowen and Rovira, 1999; Uren, 2000; Bertin et al., 2003), or absorb dissolved organic molecules released during decomposition. In both cases the molecules are oxidized and CO_2 released. Mineral nutrients resulting from the breakdown of SOM are released into the soil solution when their abundance exceeds the amount required for growth and maintenance of the microorganism involved. The CO_2 produced dissolves in soil water producing carbonic acid, which, together with the organic acids produced through microbial (and root) metabolism, solubilizes soil mineral constituents.

The amount of exudates produced by a root varies with its development, being more abundant in younger zones than in older ones. Bacteria behind the apex assimilate these substrates resulting in successive turnover waves of diminishing amplitude along the root, and an alternating immobilization and release of nutrients (Semenov et al., 1999; Zelenev et al., 2000). Considering the amount of nutrients released by plant root systems, microbial activity and succession in the root zone are certainly important steps in nutrient mobilization and cycling in soil (Azcón-Aguilar and Barea, 1992; Steinberg and Rillig, 2003; Stevenson and Cole, 1999; Toro et al., 1997).

There is growing interest in PGPRs due to the role they play in plant health and soil fertility. These organisms are often divided into two groups based on their function: those involved in nutrient cycling and mobilization, and phytostimulation, and those involved in the biocontrol of plant pathogens (see below). These two groups are not mutually exclusive. Phosphorus solubilization is a PGPR-mediated process (Richardson, 2001). Phosphorus is very reactive in soils and as a result, the concentration of phosphate ions (predominantly HPO_4^{-2} and $H_2PO_4^{-1}$) in the soil solution is very low, despite the relative abundance of total phosphorus in many soils. Because of phosphorus very high reactivity, the diffusion of phosphate ions in soil is a very slow process and phosphorus availability often limits plant growth in natural systems (Bieleski, 1973; Richardson, 2001). The problem of phosphorus availability was resolved by various soil microorganisms, which solubilize the phosphorus sequestered in the soil mineral fraction. Various PGPRs and other soil microorganisms that can solubilize P sequestered in the soil mineral fraction have been studied (Tilak and Li, 1989; Vassileva et al., 1998; Whitelaw, 2000; Richardson, 2001; Vivas et al., 2003; Gamalero et al., 2004; Barroso and Nahas, 2005).

Mycorrhizal fungi also produce soil phosphatases enzymes (Koide and Kabir, 2000) and probably excrete organic acids. However, the major contribution of AM fungi to plant P nutrition is probably related to the ability of their external mycelium to explore the soil intensively and to access microsites (Smith and Read, 1997). The phosphorus taken up by the AM mycelium throughout the soil volume can be translocated within the hyphae to plant roots. In this manner, AM fungi give plants access to phosphate ions located beyond the P-depletion zone that normally develops around the roots.

Arbuscular mycorrhizal fungi and P-solubilizing microorganisms may have synergistic or complementary effects. For example, many PGPRs are able, through the release of chelating organic acids, to solubilize sparingly soluble P, which may then be taken up by AM fungi and translocated to their host plant (Barea et al., 1983, 1997). Futhermore, AM inoculation can facilitate the establishment of both inoculated and indigenous P-solubilizing rhizobacteria (Barea et al., 2005). Moreover, AM fungi can enhance the P solubilization activity of PGPRs. For example, the bacteria *Pseudomonas aeruginosa* and *P. putida* solubilized more P when grown in the presence of *G. intraradices* than when grown alone (Villegas and Fortin, 2001).

Nitrogen also occupies a key position among those elements essential for plant and microbial growth. Living organisms have a particularly large requirement for N and it is often limiting in plant-soil systems. It appears that AM fungi have the potential to improve plant N uptake from soil. Uptake from organic sources may partly be due to the capacity of AM fungi to take up organic molecules (Hawkins et al., 2000); however, it is also likely related to the fact that AM fungi physically increase the absorbing capacity of plant root systems. Hyphae of an AM fungus proliferating in the vicinity of

organic residues may increase its host plant's competitive ability to take up NH_4^+ released through mineralization (Hamel, 2004). The ability of AM hyphae to take up and translocate NH_4^+ to plants has been clearly demonstrated (Subramanian and Charest, 1999; Tanaka and Yano, 2005; Toussaint et al., 2004). The ability of the AM fungal mycelium to exploit the soil matrix more efficiently than the roots alone may also be a significant benefit in plant N assimilation. Recently, Govindarajulu et al. (2005) investigated N transfer in the AM symbiosis using mass spectrometry and qRT-PCR approaches. The authors showed that inorganic N taken up by the AM fungus outside the roots is incorporated into amino acids, translocated from the extraradical to the intraradical mycelium as arginine, and probably transferred to the plant as ammonium.

In natural ecosystems, N is mainly incorporated into the soil through chemical and biological N_2-fixation, the latter being performed by free-living bacteria and cyanobacteria, or by plants in symbiosis with these organisms. Organic forms of N are, in turn, mineralized to ammonia by numerous bacteria and fungi, and oxidized to nitrate by nitrifying bacteria (Stevenson and Cole, 1999). Studies have suggested that the presence of AM fungi influences populations of N-transforming microorganisms, and that these interactions may modify N availability in soils. For example, the occurrence of autotrophic nitrifying bacteria in maize pot cultures was significantly higher when the pots were inoculated with either *G. mosseae* or *G. fasciculatum*, whereas ammonifying and denitrifying bacterial populations significantly decreased (Amora-Lazcano et al., 1998).

Among those studies related to the influence of AM fungi on the organisms involved in N cycling, investigations concerning N_2-fixation are the most abundant.

Phosphorus is involved in energy transport (ATP) and N_2-fixation is a very energy intensive process. Therefore, increased P uptake by a mycorrhizal plant would enhance N_2-fixation in by diazotrophic bacteria. It has been shown, using ^{15}N, that N fixation is greater in *Rhizobium*-inoculated mycorrhizal legumes than in non-mycorrhizal controls (Puppi et al., 1994; Jeffries et al., 2003). Several plant-*Rhizobium*-AM fungal combinations have been tested and all have shown increased plant development, nutrient uptake, and N_2-fixation (Requena et al., 1997). Nitrogen fixation further promotes mycorrhizal development through improved plant nutrition and growth. Plants of tripartite symbioses generally contain higher N levels and, consequently, better root and mycorrhizal development (Puppi et al., 1994; Johansson et al., 2004).

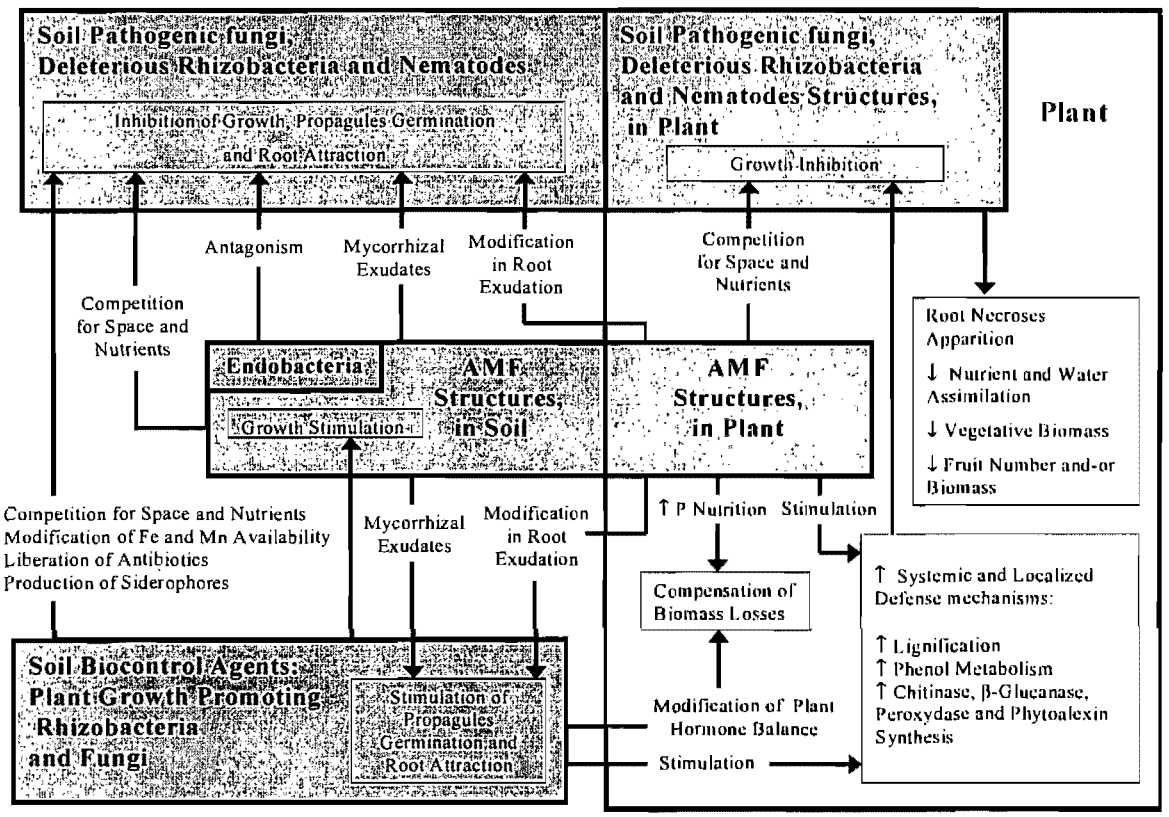
An association between *G. mosseae*, *Rhizobium meliloti*, a P-solubilizing rhizobacterium (*Enterobacter* sp.), and alfalfa plants (*Medicago sativa*) was investigated by Barea et al. (2002). In this experiment, the *Rhizobium* behaved as a mycorrhiza-helper bacteria (MHB), promoting AM establishment. In turn, AM formation increased N_2-fixation rates in *Rhizobium*-inoculated plants, compared to non-mycorrhizal controls. The dual inoculation of the AM fungus and the *Rhizobium* significantly increased microbial biomass, and N and P accumulations in plant tissues. Furthermore, dual-inoculated plants also displayed lower specific $^{32}P/^{31}P$ activity in the presence of the *Enterobacter* sp. than their comparable controls, suggesting that the mycorrhizal and bacterized plants were using P sources available through the activity of the P-solubilizing bacteria and otherwise unavailable to the plant. It therefore appears that these rhizosphere/mycorrhizosphere interactions contributed to the biogeochemical cycling of P, and increase plant fitness.

Arbuscular mycorrhizal fungi and their role in controlling soilborne plant-pathogens

Disease suppressive soils were first described over 100 years ago. These soils show minimal disease development even in the presence of virulent pathogens and susceptible plants. In these soils, propagules of the pathogens are subjected to fungistasis and the germination of their propagules is reduced. The formation of such soils was first attributed to changes in pH, and to SOM and clay contents. However, the fact that a soil's suppressive property can be eliminated by pasteurisation, or treatment with antibiotics, and can be transmitted to a non-suppressive soil by inoculation with a small amount of a suppressive soil, suggests that the responsible factor is microbial. Certain microorganisms seem to limit the development of certain pathogens, leading to the formation of specific suppressive soils (Cook and Baker, 1983; Bruehl, 1987; Mazzola, 2002). The identification and study of these soil microorganisms led to the development of a new field of research termed biocontrol, bioprotection or biological control. This was defined by Baker and Cook (1974), as *'the reduction of inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists'*. Bioprotection is an environmentally friendly and economically viable means of controlling a number of plant diseases. However, to be used efficiently, the mechanisms by which biocontrol is achieved must be identified.

Much of the research investigating the interactions between AM fungi and other soilborne microorganisms has been related to plant pathogens and was performed to

Figure 3. Diagrammatic representation of the disease inhibiting interactions betwen AM fungi, soilborne pathogens and other soil microorganisms. The plant, AM fungus, soilborne pathogens and biocontrol-related microorganisms are represented by rectangles, while interactions are indicated with arrows. AM fungi can provide bioprotection to plants directly, by modifying the signalling or defense-related biochemical pathways, and through their influence on microbial populations in the mycorrhizosphere, hampering the proliferation of pathogenic and deleterious fungi, bacteria and nematodes in the vicinity of roots.



evaluate the potential use of AM fungi as biocontrol agents (St-Arnaud and Vujanovic, 2007). Arbuscular mycorrhizal fungal mediated bioprotection has been observed in the presence of parasitic stramenopiles, especially *Phytophthora nicotianae* (Cordier et al., 1996; Pozo et al., 2002a; Trotta et al., 1996; Vigo et al., 2000), *P. fragariae* (Norman and Hooker, 2000), *Pythium ultimum* (Larsen et al., 2003; St-Arnaud et al., 1994) and *Aphanomyces euteiches* (Larsen and Bødker, 2001; Slezack et al., 1999; Thygesen et al., 2004), the pathogenic fungus *Fusarium oxysporum* (Caron et al., 1985, 1986a, 1986b, 1986c, 1986d), and nematodes (Borowicz, 2001). The level of biocontrol provided by AM fungi largely depends on the species or isolate of the latter, on the plant species or cultivar, and on the variables considered such as the biomass and the quantity and quality of the reproductive structures of both the plant and pathogen in question. However, the level of mycorrhizal colonization generally appears to be secondary. For the specific aspects of biocontrol induced by AM fungi, see reviews by Dehne, 1982; Graham, 1988; Perrin, 1990; Hooker et al., 1994; Linderman, 1994, 2000, 2001; St-Arnaud et al., 1995a; Azcón-Aguilar and Barea, 1996; Borowicz, 2001; Sharma and Johri, 2002; St-Arnaud and Elsen, 2005; St-Arnaud and Vujanovic, 2007). A great number of different combinations of plants species and cultivars, pathogens and AM fungi have been studied and in the large majority of cases, inoculation with AM fungi reduced disease development (St-Arnaud and Vujanovic, 2007), confirming the importance of considering AM fungi in plant disease management.

The mechanism first advanced as being responsible for AM fungal-mediated bioprotection was the increased P assimilation of AM plants. Mycorrhizal plants with greater nutrient uptake capacities and greater biomass would be better able to compensate for root damage caused by pathogens. However, as biocontrol was

independent of soil P availability or plant P content (St-Arnaud et al., 1994; Caron et al., 1986c; Trotta et al., 1996), it became evident that biocontrol was also due to other phenomena (Fig. 3). Among these, the stimulation of host plant defense mechanisms appears to be an important factor. The synthesis of molecules involved in plant defense reactions (in particular chitinase, β-glucanase, peroxydase and phytoalexin) is weakly elicited at the onset of mycorrhizal formation. Moreover, resistance reactions, including increased lignification and phenol metabolism stimulation, are enhanced by AM colonization (Spanu and Bonfante-Fasolo, 1988; Spanu et al., 1989; Grandmaison et al., 1993; Traquair, 1995; Gianinazzi-Pearson et al., 1996; Garcia Garrido and Ocampo, 2002). Arbuscular mycorrhizal fungal interactions with plants may activate or stimulate plant physiological pathways related to disease resistance and condition the roots to react faster when confronted with pathogens (Benhamou et al., 1994; Pozo et al., 1996, 1998, 2002b; Slezack et al., 2000; Zhu and Zao, 2004). The observed biocontrol effect may either be localized or systemic (Cordier et al., 1998; Pozo et al., 2002a).

Colonization by AM fungi also changes the quantity and quality of root exudates (Graham et al., 1981; Bansal and Mukerji, 1994; Azaizeh et al., 1995; Marschner et al., 1997; Sood, 2003; Usha et al., 2004). Citric acid was detected in the soil solution collected from the hyphal compartment of plants colonized with *Gigaspora margarita* (Tawaraya et al., 2006). The potential role of such changes on plant bioprotection was investigated by Lioussanne et al. (cf. chapter IV) using a compartmented *in vitro* system (Fig. 4). This approach permitted the collection of exudates from tomato root-organs (colonized or not with *G. intraradices*) without having the confounding effects from other rhizospheric microorganisms. When *P. nicotianae* zoospores were exposed to exudates from mycorrhizal or non-mycorrhizal mature tomato roots (Fig. 5), the former

Figure 4. Bi-compartmental Magenta box used for mycorrhizal and non-mycorrhizal tomato root exudates production (cf. chapter IV). Mycorrhizal and non-mycorrhizal transformed tomato roots were grown in a central compartment filled with gellified minimal medium (Labour et al., 2003). Roots and AM mycelia were let to grow into the peripheral compartment containing a liquid and sugarless minimal medium, three times enriched in phosphate. Medium from the peripheral compartment containing the exudates was collected and used in biotests or analysed in HPLC after filtration and freezing.

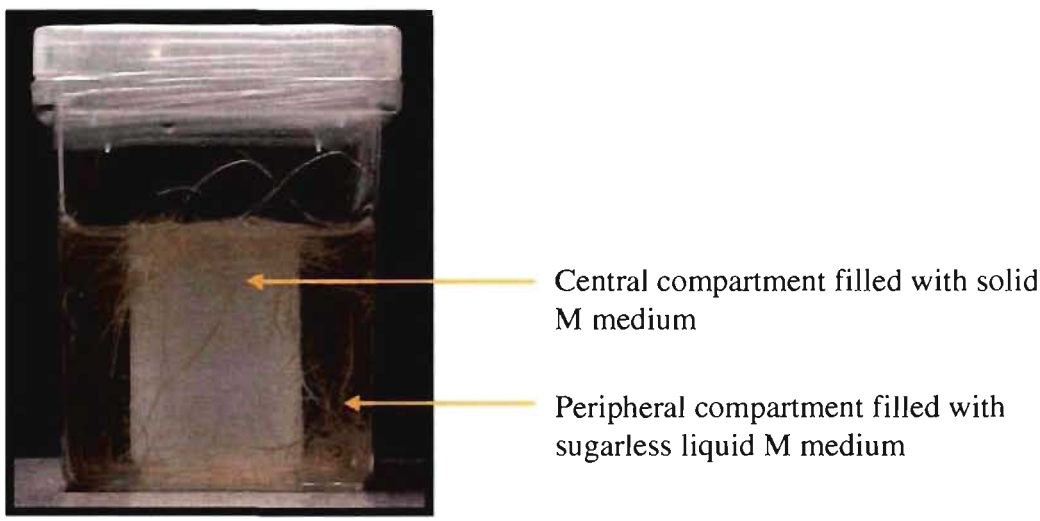
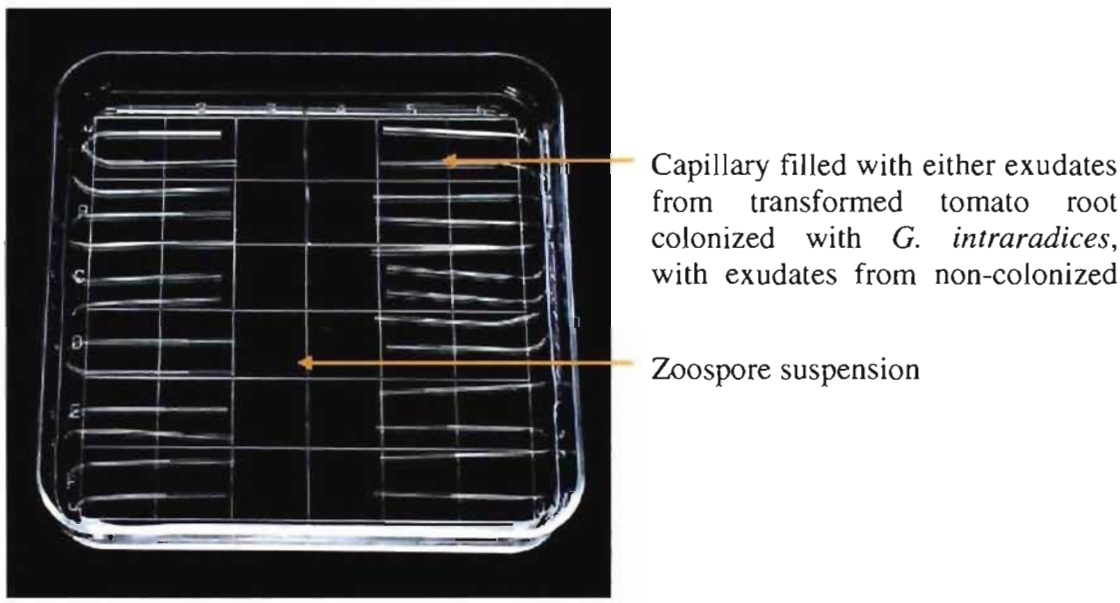


Figure 5. Capillaries filled with either mycorrhizal, non-mycorrhizal tomato root exudates or sterilized pure water (control) were exposed to *Phytophthora nicotianae* zoospore suspensions (cf. chapter IV). Attraction or repulsion of exudates was estimated by counting the number of zoospores in each capillary after killing with glutaraldehyde.



were less attractive to *P. nicotianae* zoospores than the latter. By contrast, exudates from mycorrhizal tomato roots grown in soil enhanced germination of *F. oxysporum* microconidia (Scheffknecht et al., 2006, 2007). Therefore, AM colonization would modify the composition of tomato exudates, which may lead to a change in root-microbial interaction in the soil. Filion et al. (1999) showed reduced *F. oxysporum* conidia germination in the presence of crude extracts of *in vitro* grown *G. intraradices* mycelium, while germination of conidia of the biocontrol agent *T. harzianum* was increased. Similar inhibitive effects on *in vitro* sporulation of the pathogen *P. fragariae* were observed in the presence of exudates from strawberry roots colonized by *G. etunicatum* and *G. monosporum* (Norman and Hooker, 2000). These results suggest that changes in root exudates quality and quantity affect pathogens growth directly. Furthermore, exudates of tomato roots colonized by *G. fasciculatum* were also more attractive to *Pseudomonas fluorescens* and *Azotobacter chroococcum* than exudates liberated by non-colonized roots (Sood, 2003). Therefore, *in vivo*, changes may also occur in the proliferation of other microorganisms in the rhizosphere that are able to help to reduce the proliferation of soilborne pathogens. The identification of the molecules responsible for the above effects would facilitate the development of effective AM-mediated bioprotection strategies. Although differences in the amino acid, organic acid and sugar content of mycorrhizal and non-mycorrhizal root exudates have been described, the impact of these changes on other soil microorganisms has not been tested (Sood, 2003). Modification in the production of signal molecules in mycorrhizal plants may also be involved in biocontrol. This is supported by studies showing that a mycorrhizal plant can inhibit further root colonisation by the same or different AM fungi, and that this is controlled by altered root exudation patterns (Pinior

et al., 1999; Vierheilig and Piché, 2002; Vierheilig et al., 2003; Vierheilig, 2004) and systemic signalling (Ludwig-Muller, 2000a; Vierheilig et al., 2000b; Vierheilig and Piché, 2002; Herrera Medina et al., 2003; Vierheilig, 2004b). Blumenin (Fester et al., 1999, 2002a), acacetin and rhamnetin (Scervino et al., 2005a) are secondary metabolic compounds found only in colonized roots. These molecules may be involved in the regulation of the symbiosis and in the functioning of arbuscules. Moreover, AM fungal genus-, species- and development-specific effects of the flavonoids chrysin, isorhamnetin, kaempferol, luteolin, morm and rutin were recently shown (Scervino et al., 2005b). However, this has prevented the establishment of any generalization concerning the effect of root flavonoids on AM fungi.

Similarities in the systemic regulatory mechanism of the AM association and rhizobium symbiosis were demonstrated using a split-root system (Catford et al., 2003) in which *G. mosseae* colonization suppressed *Sinorhizobium meliloti* nodulation and vice-versa. As root diseases can also be reduced systemically in mycorrhizal root systems (Pozo et al., 2002a), pathogen development may also be affected by the same regulatory mechanisms. Secondary metabolites induced by AM fungal colonization may play a significant role in the observed AM-mediated biocontrol. This hypothesis is strongly supported by the accumulation of the flavonoids rishitin and solavetivone in potato roots colonized by *G. etunicatum*. These flavonoids, which inhibit *Rhizoctonia solani* growth, only accumulated in potato roots when they were challenged by the pathogen (Yao et al., 2003).

AM fungal mediated biocontrol may also be partly due to a competition for space and nutrients between AM fungi and root pathogens. Cordier et al. (1996) showed that *P. nicotianae* and *G. mosseae* never simultaneously occupied the same tomato root

tissues. Furthermore, fatty acid signatures have been used to show the reduction in biomass and energy reserves of *G. mosseae* and *Aphanomyces euteiches* co-occupying pea roots (Larsen and Bødker, 2001). In most studies to date, plants have been pre-colonized with AM fungi prior to being inoculated with the pathogen; however, this has been shown to be unnecessary for the obtention of the biocontrol effect (Caron et al., 1986b; St-Arnaud et al., 1994, 1997). By contrast, different plant pathogens may reduce the extent of mycorrhizal colonization (Bååth and Hayman, 1983; Davis and Menge, 1980; Krishna and Bagyaraj, 1983). This again supports the occurrence of competitive interactions. Inhibition of the proliferation of deleterious rhizobacteria by mycorrhizal colonization has also reported (Garcia Garrido and Ocampo, 1988, 1989).

Synergistic interactions between AM fungi and PGPRs or other soil microorganisms may enhance bioprotection. For example, Siddiqui and Mahmood (1998) demonstrated that the combined inoculation of *G. mosseae* and *P. fluorescens* caused a greater reduction in galling and nematode reproduction than when they were used alone. Diedhiou et al. (2003) also showed a similar interaction between *G. coronatum* and the non-pathogenic *F. oxysporum* strain Fo162 in the control of *Meloidogyne incognita* on tomato.

The mechanisms used by PGPRs to protect plants against pathogens are well known: competition for space and nutrients, modification of Fe and Mn availability, liberation of antibiotics and HCN, plant growth promotion by modification of plant hormone balance, stimulation of systemic and localized plant defense mechanisms (Azcón-Aguilar and Barea, 1996; Nehl et al., 1997; Piga et al., 1997; Bowen and Rovira, 1999; Larkin and Fravel, 1999). PGPRs may act in concert or be stimulated by AM colonisation. *Paenibacillus* sp. strain B2 isolated from the mycorrhizosphere of *G.*

mosseae-colonized *Sorghum bicolor* had an antagonistic effect on *P. nicotianae in vitro* and *in vivo* (Budi et al., 1999, 2000). This suggests that the AM-mediated biocontrol of this pathogen (Cordier et al., 1996; Trotta et al., 1996; Vigo et al., 2000; Pozo et al., 2002a) is due to the AM fungus and its associated bacteria. A species of *Paenibacillus* was also frequently found in the hyphosphere of cucumber plants colonized by *G. intraradices* (Mansfeld-Giese et al., 2002), indicating that bacteria of this genus may live in close association with the AM fungal mycelium. The increase in AM fungal biomass caused by the presence of MHBs would intensify the biocontrol effect. Nonetheless, mycoparasitism by the biocontrol fungus *Trichoderma* (a PGPR) has been observed (Brimner and Boland, 2003), especially on *G. intraradices* and *G. mosseae*. Moreover, Ravnskov et al. (2002) and Larsen et al. (2003) reported a reduction of biomass of the biocontrol agent *Burkholderia cepacia* in the presence of *G. intraradices*. This highlights the need for controlled experiments prior the use of biocontrol agents in the field.

Nevertheless, management of microbial resources in the field (in particular AM fungi) is likely to be a promising avenue towards the control of plant diseases. Recently, in a large scale study on *Fusarium* crown and root rot disease in asparagus, a reduction in AM fungal biomass was shown to be one of the most significant factors associated with disease outbreaks (Hamel et al., 2005a, 2005b). In this study, *Fusarium* community structure was shown to include at least 16 species that varied in relation with climatic geographical regions, soil types, cultivars and plant tissues, but not with plant health (Vujanovic et al., 2006). Sampling site and plant age significantly influenced the AM fungal community structure, while only sampling site consistently influenced the *Fusarium* community. Diseased and healthy plants hosted similar

Fusarium and AM fungal communities (Yergeau et al., 2006), but the distribution of some *Fusarium* and AM fungal strains were largely mutually exclusive. This observation may reflect antagonism between some AM fungal and *Fusarium* isolates, and this issue is worth further investigation. Our understanding of the ecology of microorganisms within the rhizosphere has been hampered by the lack of reliable, fast and low-cost high throughput approaches required to process the number of samples in large-scale ecological studies. The increasing availability of culture-independent PCR-based technologies has greatly improved the direct detection, identification, and characterization of microorganisms within soil. Various methods such as DGGE 'denaturating gradient gel electrophoresis' (Duineveld et al., 2001; Marschner and Baumann, 2003; deSouza et al., 2004; Yergeau et al., 2005), SSCP 'single strand conformation polymorphism' (Lee et al., 1996; Brandao et al., 2002), T-RFLP 'terminal restriction fragment length polymorphism' (Tiedje et al., 1999), and real-time PCR (Filion et al., 2003a, 2003b; Harms et al., 2003) are now widely available, making it possible to investigate the highly complex multitrophic interactions of AM fungi with other soil microorganisms. These techniques are likely to refine our classical view of the roles and functions of soil microbes, and allow the optimization of the use and management of soil-inhabiting microorganisms in plant production.

Conclusion

The effects of AM fungi on soil microorganisms influence soil fertility and plant health. This influence is largely due to modifications in the quantity, quality and distribution of plant-derived C in soil. This varies with the host plant, the AM fungus or fungi involved, the other microorganisms present and the prevailing environmental conditions. AM fungi can influence those soil microorganisms mineralizing SOM, solubilizing minerals, chelating metals and fixing N. They also provide bioprotection to plants directly, by modifying the signalling or defense-related biochemical pathways, and through their influence on microbial populations in the mycorrhizosphere, hampering the proliferation of pathogenic and deleterious fungi, bacteria and nematodes in the vicinity of roots. The latter probably constitutes a large part of the bioprotective capability of the AM symbiosis. The successful management of AM fungi in agriculture and forestry could reduce the need for agro-chemicals and improve the sustainability of these production systems.

Commercial AM fungal inoculants exist in several countries, including the USA and Canada. They are primarily marketed as biofertilizers for home gardening, landscaping and for the production of certain ornamental plants. However, due to regulation specificities, they cannot currently be registrered as biopesticides (Whipps, 2004). Although AM fungi are rarely managed in crop production systems, they almost certainly contribute to crop yield. Public demand for sustainable systems increases and biotechnology evolves. Consideration of AM fungal resources in crop management can be expected to increase with the development of easy to use, effective and inexpensive inoculants. Not only will the bioproducts that are currently being tested become

valuable tools for the management of soil microbial communities in plant production systems, they will allow a more environmentally friendly approach to agriculture and forestry.

Chapitre IV

Mycorrhizal colonization with Glomus intraradices and development stage of transformed tomato roots significantly modify the chemotactic response of zoospores of the pathogen Phytophthora nicotianae

Laëtitia Lioussanne, Mario Jolicoeur & Marc St-Arnaud

Ce chapitre constitue la version finale de l'article soumis le 29 octobre 2007 à la revue Soil Biology and Biochemistry. Les résultats ont également été présentés lors des congrès indiqués ci-bas.

Lioussanne, L., M. Jolicoeur et M. St-Arnaud. 2003. Effects of exudates extracted from transformed-tomato-roots grown in bi-compartment Magenta dishes and colonized with or without *G. intraradices* on *Phytophthora parasitica* var. *nicotianae* zoospores. *Proceedings of the Canadian Society of Plant Physiologists Eastern Regional Meeting*, Montréal, QC, pp. 291.

Lioussanne, L., M. Jolicoeur et M. St-Arnaud. 2003. Effects of the alteration of tomato root exudation by *Glomus intraradices* colonization on *Phytophthora parasitica* var. *nicotianae* zoospores. *Proceedings of the Fourth International Conference on Mycorrhizae*, Montréal, QC, pp. 291.

Lioussanne, L., M. Jolicoeur et M. St-Arnaud. 2004. L'attraction des zoospores du *Phytophthora nicotianae* par les exsudats racinaires de tomate est diminuée par la colonization mycorhizienne. *Réunion annuelle de la Société québécoise de protection des plantes*, Sherbrooke, QC, pp. 34.

Lioussanne, L., M. Jolicoeur et M. St-Arnaud. (2004). Transformed tomato root exudates are less attractive to zoospores of *Phytophthora nicotianae* after colonization by the arbuscular mycorrhizal fungus *Glomus intraradices*. *Proceedings of the 75th Annual Congress of the Canadian Phytopathological Society*, Ottawa, ON, pp. 39.

Abstract

The chemotaxic response of *Phytophthora nicotianae* zoospores towards exudates from mycorrhizal and non-mycorrhizal transformed tomato roots was studied. A bi-compartmental *in vitro* system was used to grow Ri T-DNA transformed tomato roots colonized or non-colonized with the arbuscular mycorrhizal fungus *Glomus intraradices* and to collect root and mycorrhizal exudates. The root and mycorrhizal growth dynamics were first characterized in order to determine two times of exudate sampling. Exudates collected from 16-wk-old mycorrhizal roots were significantly more attractive for *P. nicotianae* zoospores than exudates from non inoculated roots. On the contrary, exudates 25× concentrated and harvested from 24-wk-old mycorrhizal roots were repulsive to zoospores compared to exudates from non-colonized roots and the water control. HPLC-MS analyses revealed a significantly higher concentration of proline and isocitrate in exudates of *G. intraradices* inoculated roots after 24 wk of growth. However isocitrate concentration was significantly higher within exudates from non-mycorrhizal roots, after 16 wk of growth. Mycorrhizal inoculation had no effect on the amounts of other amino acids and organic acids and on the sugars quantified within exudates.

Keywords

Arbuscular mycorrhizal fungi; *Phytophthora nicotianae*; bioprotection; exudate; zoospore; chemotaxy.

Introduction

Arbuscular mycorrhizal (AM) fungi have been frequently shown to reduce damages and the growth of soilborne pathogens in a wide range of mycorrhizal plant species and pathogenic organisms (St-Arnaud and Vujanovic, 2007). These fungi constitute the phylum *Glomeromycota* (Schüßler et al., 2001) and they form a mutualistic symbiosis with a large majority of the land plants, including most agricultural crop species (Harley and Harley, 1987). AM fungi are obligate symbiots and absorb carbon from the plant which in turn gains a better access to minerals and water through the fungal mycelium exploring the soil more efficiently than roots (Smith and Read, 1997; Strack et al., 2003). The understanding of the bioprotection conferred by AM fungi is still at its infancy even if several mechanisms have been proposed to be implicated in this phenomenon, particularly an increased phosphorus assimilation and changes in defence responses (cf. chapter III), that however have been shown not to occur in all cases (Trotta et al., 1996; St-Arnaud et al., 1997; Guillon et al., 2002). Among the pathogenic systems previously studied, a reduction of the growth of *Phytophthora nicotianae* in tomato roots colonized with the AM fungus *Glomus mosseae* and a concurrent decrease of diseases symptoms have been demonstrated by several authors (Cordier et al., 1996; Trotta et al., 1996; Vigo et al., 2000). Local and systemic changes in plant defence responses were described (Cordier et al., 1996, 1998; Pozo et al., 1996, 1998, 1999, 2002a), but the reduction in the number of infection loci in mycorrhizal tomato roots suggests that the pathogen may also be affected prior to root penetration (Vigo et al., 2000).

P. nicotianae is an Oomycota (Stramenopiles) presenting a wide host range and attacking more than 72 genera from 42 families of flowering plants (Satour and Butler, 1967). It causes root rot diseases leading to important yield losses in many agriculturally important crops, and spreads through asexual reproduction, at the origin of a massive liberation of zoospores. The accumulation of zoospores behind root tips and their chemotaxy towards root exudates have been studied. Quantitative assays revealed that zoospores are attracted by amino acids (especially glutamic and aspartic acids), organic acids, sugars, secondary metabolites and volatiles, depending on the tested isolate or species. Moreover, they do not take up nutrients until germination which means that receptors are implicated in their chemotaxy (Deacon and Donaldson, 1993).

Exudates which are composed of passively lost low molecular weight water soluble compounds, of secreted higher molecular weight substances which depend on metabolic processes for their release and of mucilage represent an important loss of photosynthate from roots (Lynch and Whipps, 1990; Bertin et al., 2003). Exudates condition the rhizosphere which is characterized by specific physical and chemical soil properties and a high microbial activity, and shows a specific and stable diversity that plays an important role on plant health (Bowen and Rovira, 1999). Exudates from resistant cultivars of *Cicer arietum* and of *Solanum lycopersicum* stimulated significantly less spore germination of *Fusarium oxysporum* f.sp. *ciceri* and of *F.o.* f.sp. *lycopersici* in comparison to exudates from sensitive cultivars (Mandal and Sinha, 1991; Stevenson et al., 1995). Toxic compounds would be constitutively liberated by resistant cultivars and the composition of root exudates would be of importance to determine the emergence or the inhibition of soil pathogens. Even if AM fungi are

known for increasing plant biomass, they have been shown to decrease root exudation in many studies (Graham et al., 1981; Bansal and Mukerji 1994; Azaizeh et al., 1995; Marschner et al., 1997) even if the opposite was also reported (Sood, 2003; Usha et al., 2004). Only citric acid has yet been detected as an AM fungal exudate in the soil solution from an hyphal compartment of plants colonized with *Gigaspora margarita* (Tawaraya et al., 2006).

While AM fungi are obligatory biotrophic organisms, their *in vitro* culture in compartmentalized *in vitro* systems has simplified the study of their metabolism and of their interaction with soil microorganisms (St-Arnaud et al., 1995b, 1996; Fortin et al., 2002). Taking advantage of this approach, we tested the hypothesis that colonization of tomato root with the AM fungus *G. intraradices* would modify the composition of root exudates and in this manner the chemotactic response of *P. nicotianae* zoospores. The first objective of this study was to characterize transformed tomato roots and *G. intraradices* growth dynamics in the liquid compartment of a compartmentalized Magenta dish system we conceived in order to define pertinent times of exudate collection. The second objective was to assess the effect of mycorrhizal colonization on the attractiveness of exudates towards *P. nicotianae* zoospores. Finally, the third objective was to analyze the composition of exudates in specific low molecular weight compounds (sugars, amino acids and organic acids) in order to identify changes potentially responsible for differences in zoospore chemotactic responses.

Materials and methods

Bi-compartmental Magenta dish system

Magenta dishes (Sigma, GA-7 Vessel) were split into two compartments by the introduction of a polypropylene bottle (Nalgene, 60 ml), sectioned transversally at 53 mm from the bottom, and centred inside the dish. The systems were autoclaved for 60 min, at 121°C. The central compartments were filled with 50 ml of minimal medium (M) solidified with 0.4% (w/v) gellan gum (ICN Biochemical,Cleveland, Ohio), as described in Fortin et al. (2002), while the peripheral compartments were supplied with 150 ml of modified liquid M medium containing 0.12 mM KH_2PO_4 and lacking sucrose (Jolicoeur et al., 2002).

Biological material

Ri T-DNA-transformed tomato roots (*Solanum lycopersicum* L.) line 4C4 (Labour et al., 2003), colonized or non-colonized with *Glomus intraradices* Schenck and Smith (DAOM 181 602), were grown in M medium as described in Fortin et al. (2002). *Phytophthora nicotianae* Breda de Haan (ATCC 13 196) was grown on autoclaved V8 agar medium [V8 juice (Campbell Company, Toronto, ON) 1:10 diluted in Milli-Q water (Milli-Q synthesis, RiOs™, Millipore, Mississauga, ON), 0.2% (w/v) $CaCO_3$, 1% (w/v) gellan gum, 0.005% (w/v) piramicin, 0.025% (w/v) ampicillin, 0.001% (w/v) rifampicin] at 26°C, under 16h of light and 8h of darkness per day (Tuite, 1969). In order to induce zoospores production, 3 cm^2 pieces of gel from 2-wk-old cultures were transferred in Petri dishes containing 30 ml of sterilized Milli-Q water (Milli-Q synthesis, RiOs™, Millipore, Mississauga, ON). From 3 to 5 days after, the zoospores

were released by chilling the dishes at 4°C for 30 min and warming them at 26°C, for another 30 min. The concentration of zoospores was estimated by the use of an hematocytometer and adjusted to 40 000 zoospores per ml with sterilized Milli-Q water.

Root and *G. intraradices* growth dynamics and determination of exudate sampling times

Magenta dishes were inoculated with *G. intraradices*-colonized roots or with non-colonized roots as a control. In each growth unit, a 2 cm^2 piece of gel containing 5 root apexes from a 3-wk-old transformed tomato root culture was transferred into the central compartment. In the case of *G. intraradices*-inoculated roots, the presence of mycelium and of approximately 50 spores per piece of gel was verified under a dissecting binocular at 25× magnification. The cultures were then transferred in an incubator in the dark, at 26°C. The experimental design was a split-plot with 12 harvest times randomized in the main plots and the *G. intraradices*-inoculated or non-inoculated dishes randomized in the subplots. The experiment was carried out twice with 5 blocks in the first run and 3 blocks in the second one, and each treatment was represented once per block. All roots grown into the peripheral compartment were collected weekly at wk 4 to 13, and at wk 15, 16 and 20 in the first run, and at wk 4 to 11, 14 to 16, and at wk 18, 20, 22, 24 and 30 in the second run. The central polypropylene bottle was withdrawn, the roots were cut around the edge of the bottle and the liquid from the peripheral compartment was filtrated (Nitrocellulose Filters, Ø 0.45 µl, Fisher Scientific, Ottawa, ON) to collect the remaining roots and spores. The root fresh weight was measured before roots were cut into 1-cm sections, thoroughly mixed, cleared in

10% KOH for 5 min at 90°C and stained with acid fuschin (Kormanik and Mc Graw, 1982). The percentage of root length colonized by *G. intraradices* was then determined by the use of the gridline intersect method (Giovannetti and Mosse, 1980). The total number of spores was also counted. The root growth, the percentage of root length colonized and the number of arbuscules and of spores were plotted against time to characterize the root and the mycorrhizal growth patterns within the peripheral compartment of the system. Two exudate collection times were then selected: 16 and 24 wk corresponding respectively to the exponential root growth and to the plateau phase, both with appreciable levels of mycorrhizal colonization and numbers of spores.

Collection of exudates

A new series of cultures was prepared, as described above. The experimental design was a split-plot with 18 blocks. The two harvest times were randomized in the main plots and *G. intraradices*-inoculated or non-inoculated growth systems were randomized in the subplots. After 16 and 24 wk of growth, the liquid from the peripheral compartment was withdrawn , filtrated (Fisher Scientific, Nitrocellulose Filters, Ø 0.45 µl), frozen into liquid nitrogen, lyophilized until dryness and stored at –80°C. The root fresh weight, the percentage of root length colonized by *G. intraradices* and the number of spores were assessed as described before.

Attraction of *P. nicotianae* zoospores by exudates

The following biotest was used to assess the chemotactic response of *P. nicotianae* zoospores towards exudates from roots colonized or non-colonized with *G. intraradices*. Exudate samples were rehydrated with sterilized Milli-Q water to 0.1×,

1×, 10× and 25× of the initial concentration. At 25×, the exudates were passed through mixed cellulose ester membrane filters (Millipore, 0.45 µm, 13 mm) inserted in Swinnex filters holders (Millipore, 13 mm) to remove insoluble material. One end of a capillary (Drummond Scientific Co. Micro-pipettes, 2 µl capacity) was obstructed under a flame, and the other end was introduced in the exudate solution to fill it. Capillaries filled with sterilized Milli-Q water or clarified V8 liquid medium [supernatant of V8 juice (Campbell Company) 1:10 diluted in Milli-Q water, 0.2% (w/v) $CaCO_3$, centrifuged during 20 min at 4 000 rev min^{-1})] were used as a negative and positive controls respectively. The capillaries containing the exudates or control samples were disposed in a square Petri dish (Fisher Scientific) that was then filled with 30 ml of *P. nicotianae* zoospores suspension. The optimal exposition time varied with exudate concentration and was chosen to gain a maximum number of zoospores in the capillaries. Therefore, after 30 min at 10×, 1h at 0.1× and 1×, and 2h at 25×, 2 ml of glutaraldehyde 4% were introduced in the zoospore suspension in order to stop the zoospore swimming. The number of zoospores present in each capillary was counted under a reverse-microscope at 100× magnification. Seven mycorrhizal root exudate samples from growth systems showing root colonization were chosen. For each biotest, a corresponding non-inoculated control exudate sample from the non-inoculated dish within the same block and a negative water control were also employed. Only one capillary was used as a positive control since the zoospores were then innumerable. One biotest was performed for each exudate concentration and was carried out three times per concentration for each of the seven exudate sample.

Quantification of low molecular weight compounds within exudates

All exudate samples used for the biotests and rehydrated at a 10× concentration were analyzed to determine their contents in selected sugars, amino acids and organic acids.

Glucose, fructose and sucrose were quantifed as described in Labour et al. (2003) with a Beckman Coulter HPLC system (Beckman Coulter Canada Inc., Misissauga, ON) using a pump model 126, an automatic injector model 508 and a refractive index detector. Carbohydrates were separated using the Alltech 700CH column (Alltech Canada, Guelph, ON) maintained at 85°C. The mobile phase consisted in deionized water flowing at a rate of 0.5 $ml \cdot min^{-1}$.

Amino acids were quantified by HPLC-MS (Micromass ZQ, Waters, Milford, MA) after derivatization using the Waters AccQ tag amino acids reagent (Waters) according to the procedure recommended by the manufacturer. Derivatized amino acids were separated on a Symmetry C_{18} column (150*2.1 mm, 3.5 µm; Waters) equipped with a Symmetry C_{18} guard-column (Waters). The mobile phase consisted in phases A and B composed of 10 mM ammonium acetate, pH 5.00 and 60% (v/v) acetonitrile respectively. The flow rate was set at 0.3 $ml \cdot min^{-1}$ with the following linear gradients of phase B: 0 to 8 min from 2% to 5%, 8 to 24 min to 10%, 24 to 30 min to 20%, 30 to 32 min at 20%, then 32 to 37 min to 33%. The detection was performed using a single quadrupole mass spectrometer under positive electrospray conditions (capillary voltage 3.2 kV, cone voltage 20V, source temperature 150°C and dessolvatation temperature 350°C). Quantification was performed in single ion recording (SIR) mode, based on the detection of the [M+ H]+ ion for each derivatized amino acid and then by integrating

peak areas using calibration curves for amino acids standards (20-100 μM) (all from Sigma-Aldrich Canada Ltd., ON). Cysteine could not be detected using this method and was not therefore quantified.

Organic acids were analyzed using an ionic chromatography system (Dionex Canada, Oakville, ON). They were separated using an Ion Pac AS11-HC column equipped with an Ion Pac AG11-HC pre-column and detected by a conductivity detector. Separation was achieved at a flow rate of 1.8 $ml \cdot min^{-1}$ with a gradient of KOH in deionised water as follows: the first 8 min at 2 mM, then an increase to 12 mM for 30 sec, from 8.5 to 14 min, a linear gradient from 12 to 14 mM, from 18 to 22 min, a linear gradient to 34 mM, then a final increase to 50 mM until 27 min. Standards of citric acid, isocitric acid, formic acid (Sigma-Aldrich Canada Ltd.), oxalic acid (Fisher Scientific) and succinic acid (Anachemia Science, Lachine, QC) were prepared as a mix in deionised water.

Statistical analyses

The data analyses were performed using General Linear Model procedures of SAS/STAT software, version 9.1.3 of the SAS system for Windows (SAS Institute Inc. 2004, Cary, N.C.) by ANOVA. First, the effect of *G. intraradices* inoculation on the root fresh weight was analyzed at each harvest time. The statistical model included block, *G. intraradices* inoculation and the required interaction effects. Then, the effects of *G. intraradices* inoculation and harvest time (16 or 24 wk) on root fresh weight, percent of root length bearing mycorrhizal colonization, number of *P. nicotianae* zoospores in the capillaries and concentration of the different molecules in root exudates were analyzed. The statistical model included block, *G. intraradices*

inoculation, harvest time and the required interaction effects. A posteriori comparisons of means were performed by Turkey's studentized range tests at a 5% level of significance. In the analysis of zoospores counts, the *G. intraradices* inoculation treatment corresponded to exudates collected from mycorrhizal roots, from non-mycorrhizal roots or water as a negative control. A log transformation of the zoospores counts for 10× and 25× exudate concentrations was performed before analysis to normalize skewed distributions before ANOVA. Log transformations of arginine, asparagine, lysine and proline concentrations were also carried out for the same reason.

Results

Root and *G. intraradices* growth dynamics and determination of exudate sampling times

Root biomass in the peripheral compartment of the Magenta dish systems increased according to two phases (Fig. 6). It first displayed an exponential growth profile followed by stabilization in a plateau phase after 18 wk of growth. For roots inoculated with *G. intraradices*, the specific growth rate was μ=0.211 d^{-1} (SE ±0.032, with a coefficient of determination R^2 =0.832 ±0.062), and for non-inoculated roots μ=0.184 d^{-1} (SE ±0.018, R^2 =0.811 ±0.031). The maximum mean of root fresh weight was 0.88 g for non-inoculated roots at wk 22 and 0.91 g for roots colonized with *G. intraradices* at wk 24. The inoculation of *G. intraradices* did not significantly change the specific growth rate nor of the root fresh weight at any harvest time, except at 18 wk where the fresh weight was significantly higher in *G. intraradices*-inoculated roots ($P<0.005$).

Root colonization with *G. intraradices* was detected in the peripheral compartment after 10 wk of growth (Fig. 7). The percentage of root length bearing mycorrhizal structures increased to a mean of 8.5% ±3.2 after 24 wk and never exceeded 15%. Spores were first observed at wk 10 and their number increased to a mean of 105 ±82.9 at wk 16 and never exceeded 500 per peripheral compartment (Fig. 8). The arbuscules were first detected 14 wk after inoculation while the first vesicles were detected only after 30 wk (data not shown). No mycorrhizal structures were found within growth systems not inoculated with *G. intraradices*.

Figure 6. Root fresh weight of Ri T-DNA transformed tomato roots collected from the peripheral compartment of split Magenta dishes. The roots were colonized with *G. intraradices* (▲) or non inoculated (■). Error bars indicate error standard of the mean.

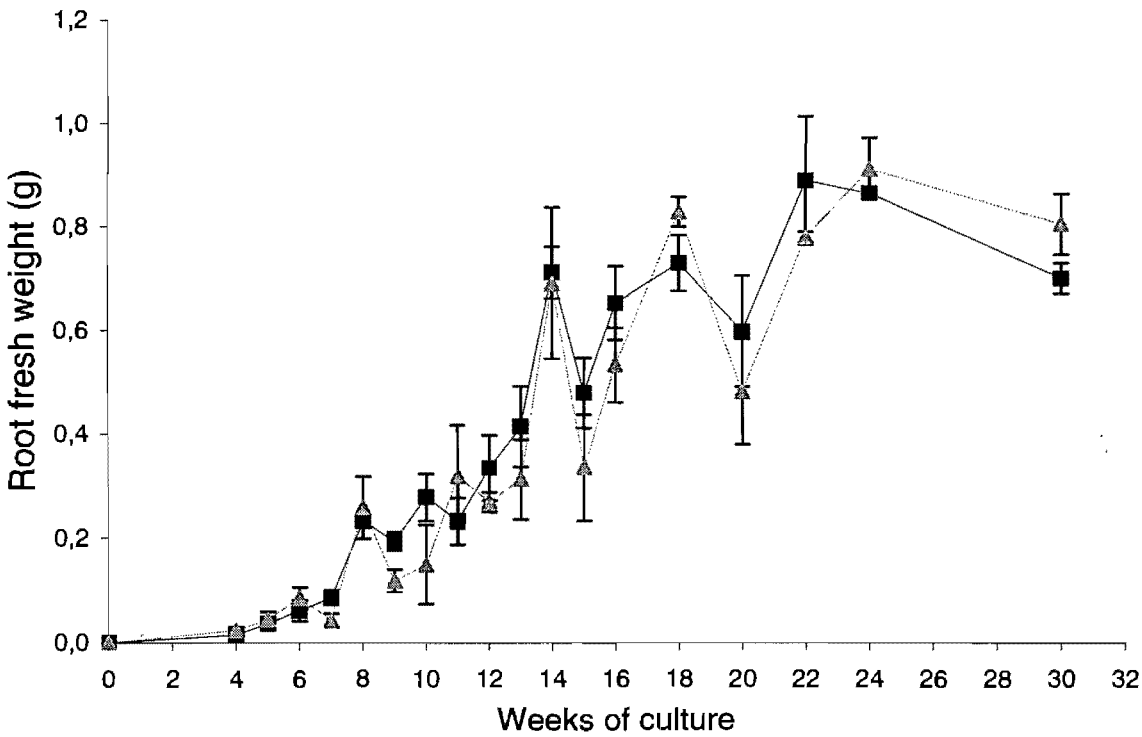


Figure 7. Percentage of colonization of transformed tomato roots with *G. intraradices* in the peripheral compartment of split Magenta dishes. Error bars indicate error standard of the mean.

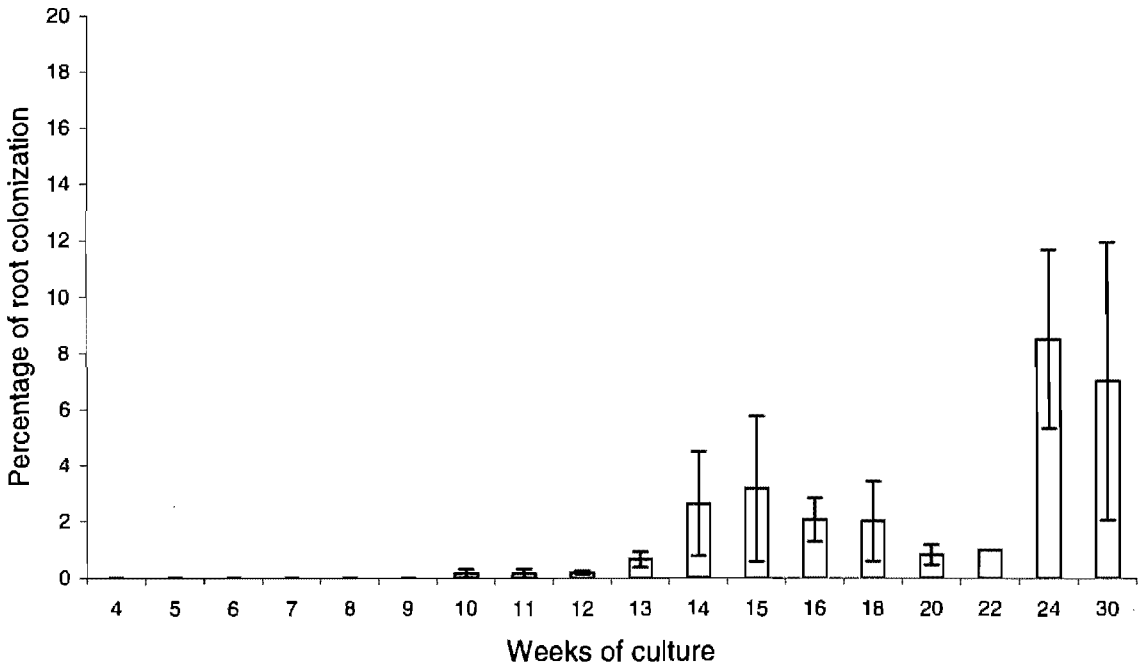
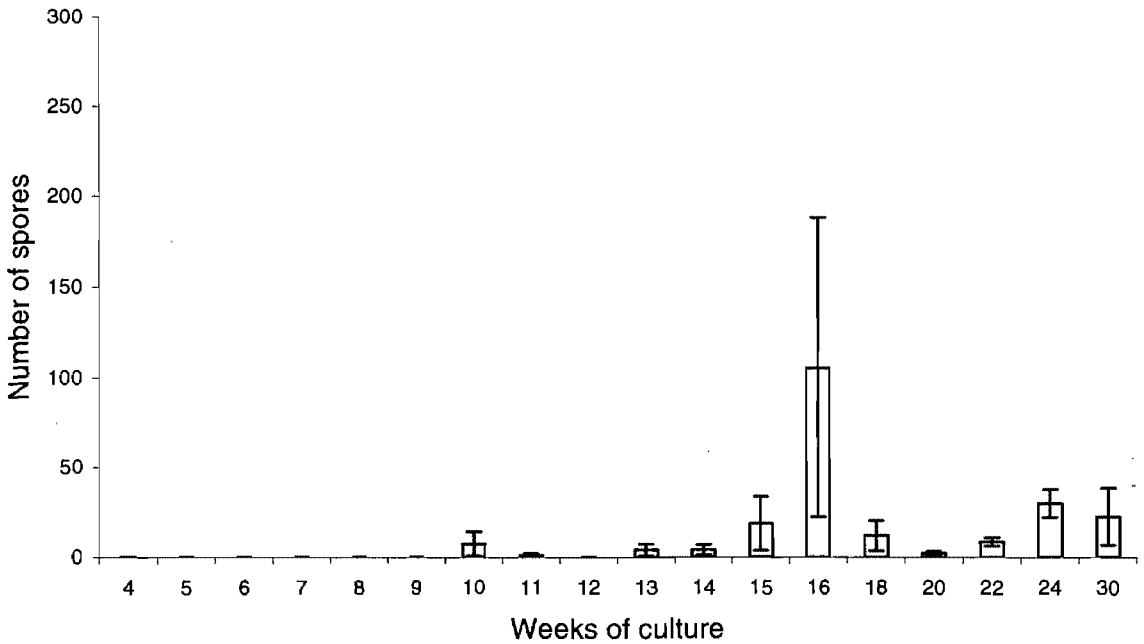


Figure 8. Number of spores present in the peripheral compartment of split Magenta dishes. Error bars indicate error standard of the mean.



Differential attraction of *P. nicotianae* zoospores by exudates

Inoculation of *G. intraradices* did not modify the root fresh weight in the peripheral compartment, which was significantly higher ($P<0.05$) after 24 wk of growth (0.836 g ±0.064) than after 16 wk (0.647 g ±0.061) (data not shown). There was no significant interaction between mycorrhizal inoculation and harvest time. There was no effect of harvest time on the percent of root length colonized (with a mean of 2.4% and a maximum value inferior to 5%) nor on the spore number (with a mean of 16.1 and a maximum that never exceeded 100), in the peripheral compartment (data not shown). No mycorrhizal structures were still detected within growth systems non-inoculated with *G. intraradices*.

The V8 positive control always attracted a higher and innumerable quantity of zoospores than other treatments. At a concentration of 0.1×, there was no significant difference in the number of zoospores attracted by exudates from *G. intraradices*-inoculated roots and exudates from non-inoculated roots, while both attracted significantly more ($P<0.0001$) the zoospores than the negative water control (Table II). As there was a significant interaction ($P<0.001$) between inoculation and harvest time at exudate concentrations other than 0.1×, the number of *P. nicotianae* zoospores present in the capillaries was analyzed separately for each harvest time.

At 16 wk, the number of zoospores was not significantly different in capillaries filled with exudates from mycorrhizal and non-mycorrhizal roots, except at 10× concentration where *G. intraradices*-inoculated exudates contained significantly more zoospores ($P<0.005$) than non-inoculated exudates and the water control. At 1× concentration, both *G. intraradices*-inoculated and non-inoculated exudates attracted

Table II. Effect of mycorrhizal colonization and harvest time on the number of zoospores of *P. nicotianae* counted in capillaries filled with different concentrations of tomato root exudates

		Inoculation treatment[1, 2]		
Concentration	Harvest time	Non-inoculated	*G. intraradices*	Water control
0.1×	16 wk	1 005.8 a, x	1 204.4 a, x	852.3 b, x
	24 wk	1 119.5 a, x	1 119.0 a, x	376.9 b, x
1×	16 wk	961.0 a, x	1 334.9 a, x	379.6 b, x
	24 wk	794.6 a, x	631.1 ab, y	341.0 b, x
10×	16 wk	174.5 b, x	408.5 a, x	93.2 b, x
	24 wk	82.5 ab, x	35.8 b, y	104.5 a, x
25×	16 wk	84.6 a, x	750.1 a, x	210.2 a, x
	24 wk	144.0 a, x	11.1 b, y	212.1 a, x

[1] Exudates collected from 16 or 24 wk-old transformed tomato roots either colonized with *G. intraradices* or non inoculated, or sterilized Milli-Q water as a negative control.

[2] There was a significant interaction between inoculation treatment and harvest time ($P<0.001$) at 1×, 10× and 25× concentrations. Within each concentration, for each harvest time, means with different letters (a, b) were significantly different between inoculation treatments ($P<0.05$), whereas for each inoculation treatment, means with different letters (x, y) were significantly different between harvest times ($P<0.005$).

significantly more ($P<0.001$) the zoospores than the negative water control, whereas no significant difference was observed between the treatments at 25× concentration.

At 24 wk, capillaries filled with exudates from mycorrhizal roots always led to a reduced zoospore massing in comparison to capillaries filled with exudates from non-inoculated roots, the difference being progressive at 1× and 10× concentrations, while highly significant at 25× concentration ($P<0.005$). At 25× and 10× concentrations, capillaries with *G. intraradices*-inoculated exudates were also significantly less attractive than water ($P<0.005$ and $P<0.05$ respectively). On the contrary, at 1× concentration, the number of zoospores was significantly higher in capillaries filled with non-inoculated exudates ($P<0.05$), and similar at 10× and 25× concentrations than in capillaries supplied with water.

On the other hand, harvest time had no influence on the number of zoospores accumulated in capillaries containing non-inoculated root exudates or water, whereas zoospores were significantly less numerous ($P<0.005$) at 24 wk than at 16 wk in capillaries filled with exudates from *G. intraradices*-inoculated roots, at all concentrations except for 0.1×.

Quantification of low molecular weight compounds within exudates

There was more fructose than glucose after 16 and 24 wk of growth in the peripheral compartment while no sucrose was detected at any harvest time (Table III). No significant difference between *G. intraradices*-inoculated and non-inoculated exudates was measured in the amount of sugars that constituted the dominant compounds quantified. Exudates collected from 16-wk-old cultures contained

Table III. Composition of exudates collected from transformed tomato roots colonized with *G. intraradices* or non inoculated after 16 or 24 wk of growth

Compound	Concentration (µM)[1]			
	Inoculation treatment[2]		Harvest time	
	Non-inoculated	*G. intraradices*	16 wk	24 wk
Sugars				
Fructose	2 802.30	2 857.85	4 143.49	1 516.66**
Glucose	920.48	933.77	1 539.52	314.72*
Sucrose	ND [2]	ND	ND	ND
Amino acids				
Alanine	0.43	0.64	0.66	0.41
Arginine	2.47	2.53	2.00	3.00
Asparagine	7.98	13.40	10.81	10.59
Aspartic acid	0.87	1.04	0.86	1.06
Glutamine	7.79	14.79	4.91	17.68*
Glutamic acid	5.20	5.90	2.85	8.25*
Glycine	4.91	6.08	5.78	5.20
Histidine	ND	ND	ND	ND
Isoleucine	0.34	0.50	0.32	0.51
Leucine	0.18	0.26	0.21	0.23
Lysine	1.96	2.37	2.37	1.75
Methionine	ND	ND	ND	ND
Phenylalanine	0.11	0.13	0.11	0.13
Serine	0.53	0.52	0.57	0.47
Threonine	0.26	0.31	0.33	0.24
Tryptophane	0.11	0.12	0.11	0.12
Tyrosine	ND	ND	ND	ND
Valine	0.38	0.49	0.44	0.43
Organic acids				
Citric acid	ND	ND	ND	ND
Formic acid	63.33	62.19	83.94	41.52*
Oxalic acid	20.30	20.48	25.15	15.63**

[1] Within inoculation treatments or harvest times, means followed by * or ** were significantly different at respectively $P<0.05$ or $P<0.005$. ND : non detected.

[2] Exudates collected from transformed tomato roots either colonized with *G. intraradices* or non inoculated.

significantly ($P<0.05$) less glutamine and glutamic acid and more fructose, glucose, formic and oxalic than exudates collected at 24 wk. As there was a significant interaction between inoculation treatment and harvest time ($P<0.05$) for the concentration of proline, isocitric and succinic acids, these data were analysed by level of each treatment. More isocitric acid was quantified within exudates from non-mycorrhizal than from mycorrhizal roots at 16 wk while the opposite was measured at 24 wk ($P<0.05$)(Table IV). At 24 wk, exudates from mycorrhizal roots contained significantly more proline ($P<0.005$) while at 16 wk, mycorrhizal inoculation had no effect on the proline content of exudates. Within exudates from mycorrhizal roots, the concentration of proline was also significantly higher at 24 wk than at 16 wk ($P<0.005$), while within exudates from non-inoculated roots, the concentration of isocitric and succinic acids were lower at 24 wk than at 16 wk ($P<0.05$). No significant difference in the other amino acids and organic acids composition was detected between both types of exudates.

Table IV. Concentration of proline, isocitric acid and succinic acid in exudates collected from transformed tomato roots colonized with *G. intraradices* or non inoculated after 16 or 24 wk of growth

Compound	Concentration (μM)[1]			
	16 wk		24 wk	
	Non-inoculated	*G. intraradices*	Non-inoculated	*G. intraradices*
Amino acid				
Proline	0.22	0.27	0.25	0.76**
Organic acids				
Isocitric acid	26.78	6.95*	2.33	6.52*
Succinic acid	26.36	20.69	15.07	23.10

[1] As there was a significant interaction between the inoculation treatment and harvest time ($P<0.05$), data for proline, isocitric and succinic acids were analysed by level of each factor. Within harvest time, means followed by * or ** were significantly different at respectively $P<0.05$ or $P<0.005$.

Among *G. intraradices*-inoculated roots, the concentration of proline was significantly higher at 24 wk than at 16 wk ($P<0.005$).

Among non-inoculated roots, the concentration of isocitric and succinic acids were significantly higher at 16 wk than at 24 wk ($P<0.05$).

Discussion

We showed here that mycorrhizal colonization and development stage of transformed tomato roots significantly modified the chemotrophic response of *P. nicotianae* zoospores to root exudates.

The *in vitro* bi-compartmental Magenta dish system used here permitted the easy collection of exudates liberated by transformed tomato roots inoculated or not with *G. intraradices* without interference from other soil microorganism, which is difficult to achieve in soil culture. The specific growth rate of the Ri T-DNA transformed tomato root line 4C4 developing within the peripheral compartment (μ=0.198 d^{-1}) was slightly higher than the value previously obtained in Petri dishes (μ=0.138 d^{-1}) (Labour et al., 2003), probably because of the higher availability of nutrients and/or gas diffusion. As in Petri culture, inoculation of *G. intraradices* did not modify root growth rate nor root biomass compared to non-inoculated roots. Root colonization as well as the spore number were also similar to the ones previously measured in Petri culture (Diop et al., 1992; Labour et al., 2003). This system proved therefore to be appropriate to compare the effect of *G. intraradices*-inoculated and non-inoculated root exudates on the behavior of *P. nicotianae* zoospores. Analyses of the root growth pattern permitted to define two harvest times: at the root exponential growth phase at 16 wk and while root growth stabilized at 24 wk.

Exudates collected from actively growing mycorrhizal roots were more attractive than exudates liberated by non-mycorrhizal roots and than the water control. At this stage of root development, the more exudates were concentrated, the less they were attractive comparing to water. Alternatively, while roots stopped growing, concentrated

exudates collected from *G. intraradices*-colonized roots were clearly repulsive towards the zoospores as they were less attractive than water and exudates from non-inoculated roots. The exudate solution is complex and may contain both attractive and repulsive molecules. The concentration of these molecules may change during root and mycorrhizal development and then influence differently the chemotaxy towards zoospores. For example, the accumulation of the secondary metabolite blumenin (4{3-O-[(2'-O-β-glucopyranosyl]-butyl}-3,5,5-trimethyl-2-cyclohexen-1-one) within roots of barley and wheat colonized with *G. intraradices* has been shown to be low when roots were 2-wk-old, reaching a maximum level when roots were 3-4-wk-old and being detected only in trace amounts when roots were older than 5 wk (Fester et al., 1999). The following hypothesis may explain our results. First, a greater number of attractive molecules would be liberated by *G. intraradices*-colonized roots during active growth while the opposite would occur at maturity. Concurrently, repulsive molecules, probably secondary metabolites, would accumulate while roots and the AM fungus are aging. The liberation of these repulsive compounds would be more important in mycorrhizal roots. Finally, at high concentration, attractive molecules may become unattractive or repulsive, especially at 25×. For a matter of fact, Cahill and Hardham (1994) and Halsall (1975) observed that the maximum attractiveness of glutamic acid towards *P. nicotianae* and *P. cinnamomi* zoospores was at 1 mM concentration. Below or over this concentration, attraction was largely reduced, or even absent.

Our results strongly suggest that tomato colonization with *G. intraradices* would modify root exudates composition that may directly impact the pathogen within the soil in the critical steps of its proliferation, before infection. The asexual reproduction is

crucial for the proliferation of pathogens. If a large number of zoospores contacts the roots, they will be massively infected. The plant defence mechanisms will be overcome and new asexual reproduction cycles will occur, leading to an exponential increase of infection. Conversely, if smaller amounts of zoospores are attracted by the roots, plant defence responses will be more efficient, the intensity of the next asexual reproduction cycles of the pathogen will be reduced and the disease outbreak delayed or prevented (Erwin and Ribeiro, 1996). Therefore, modification of the composition of root exudates would be implicated in the biocontrol observed in plants previously colonized with AM fungi. Filion et al. (1999) previously showed a reduced *F. oxysporum* conidia germination in crude extracts of *in vitro* grown *G. intraradices* mycelium. Analogous inhibitive effects were observed with exudates liberated by strawberry roots colonized by *G. etunicatum* and *G. monosporum* on the pathogen *P. fragariae* sporulation *in vitro* (Norman and Hooker, 2000). On the other hand, microconidia germination of *F. oxysporum* f. sp. *lycopersici* (*Fol*) was more than doubled in the presence of root exudates from tomato plants colonized with *G. mosseae* compared with exudates from non-mycorrhizal plants. The more the tomato plants were colonized by the AM fungus, the more microconidia germination was increased (Scheffknecht et al., 2006), suggesting a relation between the level of root colonization and the alteration of exudation pattern. Similar stimulatory effects were exhibited by the root exudates of twelve *Fol* non-host species from eight plant families, showing that mycorrhizal-induced changes in the root exudates were unrelated to the *Fol* status of the plant (Scheffknecht et al., 2007). Root exudation modification by AM colonization could also indirectly impact the pathogen proliferation by stimulating other soil microbial inhabitants that can compete with the pathogenic organism. Sood (2003) proved a

stronger attraction of *Azotobacter chrococcum* and *Pseudomonas fluorescens* by exudates collected from tomato roots colonized by *G. fasciculatum* than by exudates collected from non-colonized roots. Direct and indirect effects of exudates liberated by colonized roots could synergistically hamper the proliferation of *P. nicotianae* within the soil and prevent the plant infection. Then, the local and systemic defence responses previously observed after tomato colonization with *G. mosseae* would intervene in the reduction of the pathogen proliferation within the roots (Cordier et al., 1996, 1998; Pozo et al., 1996, 1998, 1999, 2002a).

We compared the accumulation of sugars, amino acids and organic acids within the root vicinity at its exponential growth and after its growth stabilization. Among the 27 different quantified molecules, only isocitric acid and proline amounts were significantly different between mycorrhizal and non-mycorrhizal root exudates, while fructose, glucose, glutamine, glutamic acid, proline, formic acid, isocitric acid and oxalic acid concentrations changed with root aging. Here, proline accumulated largely in exudates from mature mycorrhizal roots and may have contributed to the pathogen zoospores repulsion observed. Proline has been shown to be attractive towards *P. cactorum*, *P. capsici*, *P. cinnamomi*, *P. citrophthora* and *P. palmivora* zoospores but significantly less than aspartic acid, glutamic acid, arginine, leucine and methionine (Khew and Zentmyer, 1973). Proline is known to accumulate in plants, especially within the shoots, under abiotic stress conditions (Kuznetsov and Shevyakova, 1999; Rai, 2002; Kishor et al., 2005). Leaves of tomato accumulated proline in response to *P. nicotianae* infection (Grote and Claussen, 2001) as did the cortex of *Threbroma cacao* after infection with *P. megakarya* (Omokolo et al., 2002). Arbuscular mycorrhizal colonization was shown to increase (Vazquez et al., 2001; Diouf et al., 2005; Wu and

Xia, 2006) or not (Pinior et al., 2005) proline content within host tissues. Proline was detected within exudates from non-mycorrhizal tomato exudates but not within exudates from tomato colonized with *G. fasciculatum* (Sood, 2003). Isocitrate was also more concentrated within mature mycorrhizal root exudates than within those liberated by non-mycorrhizal roots, while the opposite was observed with actively growing roots. The differential accumulation of this organic acid alone does not coincide with the differential attractiveness of exudates from either mycorrhizal or non-mycorrhizal roots towards *P. nicotianae* zoospores. As far as we know, no data are available concerning the attractiveness of isocitrate towards Oomycètes zoospores. Sucrose, dextrose, fructose, rhamnose and maltose were shown to be largely attractive to zoospores of *P. nicotianae* at a 1% concentration (Dukes and Apple, 1961). Alternatively, no amino acid or sugar at 50 mM concentration was found to attract *P. nicotianae* zoospores, except asparagine and glutamine that were weakly attractive, while a large number of the same molecules, especially asparagine, glutamine, leucine and methionine, were largely attractive to zoospores of *P. drechsleri* and *P. cryptogea* (Halsall, 1975). In most studies, the low molecular weight compounds that were the most attractive towards Oomycètes zoospores were glutamic and aspartic acids (Deacon and Donaldson, 1993; Cahill and Hardham, 1994), even if other amino acids, sugars and organic acids were also attractive depending on the isolate or species studied.

As exudates tend to be repulsive at maturity, it is plausible that they would accumulate secondary metabolites or other high molecular weight molecules. Production of the glycoprotein glomalin by AM fungi hyphae has been well demonstrated (Wright et al., 1996). While glomalin can represent up to 5% of total C and N in soil (Rillig et al., 2001), as far as we know there is no studies available on the

effect of glomalin on soil microorganisms. The systemic autoregulation of mycorrhizal colonization (Vierheilig et al., 2000b) has been shown to happen through altered root exudation (Vierheilig et al., 2003). The flavonoids acacetin and rhamnetin (Scervino et al., 2005a), the carotenoid-derived isoprenoids blumenin, mycorradicin and nicoblumin (Fester et al., 1999; 2005; Vierheilig et al., 2000a) and the biotic and abiotic stresses related hormone jasmonic acid (JA) (Hause et al., 2002; Isayenkov et al., 2005; Stumpe et al., 2005) have been shown to accumulate in mycorrhizal roots. The application of JA on *Tropaeolum majus* and *Carica papaya* leaves highly suppressed root colonization with *G. mosseae* (Ludwig-Müller et al., 2002). The overexpression of the gene *MtAOC1* coding for the AOC enzyme involved in JA synthesis enhanced root JA levels but decreased root colonization (Hause et al., 2007). Blumenin applied to barley split-root systems resulted in systemic suppression of colonization of already mycorrhizal roots (Strack and Fester, 2006). Moreover, some carotenoid-derived compounds isolated from maize exudates have been shown to be responsible for the inhibition of *F. oxysporum* f. sp. *melongenae* spore formation induced by maize plants (Park et al., 2004). The above cited molecules would be involved in the regulation of the mycorrhizal symbiosis and may constitute good candidates responsible for the inhibition of *P. nicotianae* proliferation which has been shown to be systemically regulated in mycorrhizal root systems (Cordier et al., 1998; Pozo et al., 2002a; Vigo et al., 2000).

In conclusion, our results suggest that exudates from actively growing roots colonized with *G. intraradices* are attractive to zoospores of *P. nicotianae*, while mature mycorrhizal roots are repulsive and accumulate proline. Root colonization with AM fungi would change root exudate composition and in this manner may limit the

expansion of pathogens within the soil which would contribute to the biocontrol conferred to AM fungi. Nonetheless, these results were obtained in artificial conditions with exudates collected from Ri T-DNA transformed roots. Further experiments will be required to test whether these modifications happen in more natural conditions and really affect *P. nicotianae* infection. It is however likely that various mechanisms contribute simultaneously with their relative impact varying with the combination of plant-pathogen-AM species and environmental conditions.

Acknowledgements

We would like to thank Steve Hisiger for the HPLC analyses and the undergraduate students Sébastien Loussouarn and Anne Keough for their excellent technical assistance. This work was supported by an NSERC grant allocated to M. St-Arnaud and a FQRNT team grant to M. Jolicoeur and collaborators.

Chapitre V

The inhibition of Phytophthora nicotianae infecting tomato roots induced by the arbuscular mycorrhizal fungi Glomus mosseae and G. intraradices would not be related to root exudation modification

Laëtitia Lioussanne, Mario Jolicoeur & Marc St-Arnaud

Les résultats de ce chapitre ont été présentés lors des congrès indiqués ci-bas et seront prochainement soumis afin de publication dans une revue internationale.

Lioussanne, L., M. Jolicoeur et M. St-Arnaud. 2006. The inhibition of *Phytophthora nicotianae* induced by AMF is not related to tomato root exudation transformation. *Fifth International Conference on Mycorrhiza*, Granada, Spain, pp. 249.

Lioussanne, L., M. Jolicoeur et M. St-Arnaud. 2006. Is the inhibition of *Phytophthora nicotianae* in tomato roots induced by arbuscular mycorrhizal fungi related to changes in root exudation? 2006 *Joint Meeting of The American Phytopathological Society - The Canadian Phytopathological Society - Mycological Society of America*, July 29– August 2, 2006, Québec, QC, pp. 175. Can. J. Plant Pathol. 28: 357.

Abstract

We studied the impact of root exudation modification induced by root colonization with the arbuscular mycorrhizal fungi (AMF) *Glomus mosseae* and *G. intraradices* on the infection of the soilborne pathogen *Phytophthora nicotianae* on tomato plants. Tomato plants were grown in a compartmentalized soil system and were either submitted to mycorrhizal colonization or to application of exudates from mycorrhizal tomato plants, with the corresponding negative controls. Three weeks after planting, the plants were inoculated (or not, as a control) with *P. nicotianae* growing from a compartment inoculated with this pathogen and placed at an equal distance to every plant. At harvest, *P. nicotianae* proliferation was significantly reduced in roots colonized with *G. mosseae* or *G. intraradices* than in non-colonized roots. Conversely, the pathogen biomass was similar in roots supplied with exudates collected from mycorrhizal and non-mycorrhizal roots. Root fresh weight and shoot dry weight were increased in plants inoculated with *P. nicotianae* while the percentage of root necrosis was not significantly different between every treatment. These results suggest that the biocontrol induced by *G. mosseae* and *G. intraradices* on the disease provoked by *P. nicotianae* on tomato plants was not related to a modification in root exudation but rather to other mechanisms.

Keywords

Arbuscular mycorrhizal fungi, biocontrol, *Phytophthora nicotianae*, exudates, tomato

Introduction

The use of biocontrol strategies represents a good alternative to chemical pesticides which have become more and more restricted and have often been shown to be inefficient, to promote pathogen resistance and to cause food, soil and water contaminations. Understanding the mechanisms of bioprotection is however essential in order to use their full potential and to control the possible side effects on plant physiology and on other beneficial soil microorganisms.

Phytophthora nicotianae is an Oomycota showing a huge host range, infecting more than 72 genera from 42 plant families (Satour and Butler, 1967). This pathogenic species is at the origin of root rot diseases responsible for large yield losses in many important crops, including tomato (Erwin and Ribeiro, 1996). Under hot and wet conditions, mycelium spread is particularly favored by asexual reproduction responsible for a massive liberation of zoospores. Attraction of *Phytophthora* spp. zoospores towards the host roots is stimulated by root exudates (Deacon and Donaldson, 1993; Hickman, 1970; Ho and Hickman, 1967a; 1967b). Spore encystment, germination and formation of appressoria then follow soon after permitting host tissues infection. A positive chemotropism of germ tubes towards root exudates has also been shown (Carlile, 1983; Deacon and Donaldson, 1993; Zentmyer, 1970). After the initial infection, other cycles of asexual reproduction rapidly appear, leading to a massive root infection and to the apparition of disease symptoms (van West et al., 2003). Controlling the attraction of this pathogen is thus crucial to allow the induction of efficient plant defense mechanisms and, in this manner, significantly limit pathogen infection and its harmful effects on plant growth.

Arbuscular mycorrhizal fungi (AMF) have been shown to reduce disease development in a wide number of plant-pathogen associations (St-Arnaud and Vujanovic, 2007), including the damages caused by *P. nicotianae* in tomato plants colonized by *Glomus mosseae* which has been largely described (Cordier et al., 1996; Pozo et al., 2002a; Trotta et al., 1996; Vigo et al., 2000). Trotta et al. (1996) postulated that the better assimilation of P in plants colonized with *G. mosseae* would not be implicated in disease reduction whereas the change in activity or the induction of new isoforms of defense-related enzymes after mycorrhizal colonization were shown and suggested to be key factors limiting the pathogen proliferation within host roots (Cordier et al., 1996; 1998; Pozo et al., 1998; 1999; 2002a). Nonetheless, Vigo et al. (2000) reported that colonization with *G. mosseae* clearly reduced the number of infection loci formed by *P. nicotianae* on tomato roots, and stressed that the primarily factor responsible for determining the degree of necrosis was the number of infection loci. They suggested that mechanisms affecting the pathogen ability to penetrate roots such as modified chemotaxy resulting from a change in root exudation, or a mycorrhiza-related alteration of soil microbial communities may be involved in the biocontrol mediated by *G. mosseae*.

Moreover, AM colonisation has been shown to change the amount and quality of host root exudates (Azaizeh et al., 1995; Bansal and Mukerji 1994; Graham et al., 1981; Kapoor et al., 2000; Marschner et al., 1997; Sood, 2003). We previously investigated the impact of these changes on the chemotaxy of *P. nicotianae* zoospores, using a compartmented *in vitro* system (cf. chapter IV), that permitted to eliminate the effects of other soil microorganisms than AMF (Fortin et al., 2002; St-Arnaud et al., 1996). We showed that colonization with *Glomus intraradices* and root development stage

significantly modify the chemotactic response of zoospores. Especially, exudates collected from mature mycorrhizal roots were less attractive (and were even repulsive) to zoospores than exudates from non-mycorrhizal roots.

The objective of the present study was to test, in non-axenic soil conditions, if the biocontrol mediated by AMF on tomato plants subjected to *P. nicotianae* occurs through modification of root exudation. The hypothesis was that root exudation changes induced by mycorrhizal colonization inhibit root attraction of *P. nicotianae* then reducing root infection and disease symptoms in comparison to non-mycorrhizal plants. Tomato plants were grown individually in a compartmentalized soil microcosm and supplied with exudates from mycorrhizal plants or submitted to mycorrhizal colonization, with the negative corresponding controls. Plants were then placed at an equal distance to a compartment inoculated with *P. nicotianae*. Tomato root infection and diseases symptoms were then compared between every treatment. Two AMF species were studied : *G. intraradices* Schenck and Smith and *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe.

Materials and methods

Experimental design

Using the compartmentalized microcosm described below, twelve tomato plants were, in a first step, either submitted to application of exudates from mycorrhizal tomato plants or to direct root colonization with AMF, with the corresponding negative controls. Thus, half of the plants was supplied with sterilised pure water (E-) and either colonized with *G. intraradices* (Gi), with *G. mosseae* (Gm) or not colonized (G-). Two weeks after planting, the other half of the plants received daily 2 mL of a standardized tomato root exudate solution collected from plants colonized with *G. intraradices* (E^{Gi}), *G. mosseae* (E^{Gm}) or not colonized (E^{G-}). Three weeks later, in a second step, plants were either inoculated (P+) or non inoculated (P-) with *P. nicotianae*. Therefore, the experiment included the twelve following treatment combinations: E^{G-}G-P-, E^{Gi}G-P-, E^{Gm}G-P-, E-G-P-, E-GiP-, E-GmP-, E^{G-}G-P+, E^{Gi}G-P+, E^{Gm}G-P+, E-G-P+, E-GiP+, E-GmP+. The experimental design was a split-plot with the six mycorrhizal inoculation/exudate application treatments randomized in the main plots, and *P. nicotianae* inoculation treatments randomized in the subplots. The experiment included two blocks that each contained two replicates per treatment. The second block was started three weeks after the first one.

Biological material and growth conditions

The growth substrate was composed of a mixture of 2:2:1 (v/v) sandy loam soil collected from the 0-15 cm depth of a non chemically treated field located in the Montreal Botanical Garden (pH 6.9, 56% sand, 36 % silt, 8% clay, 9% organic matter,

126 mg P kg^{-1}, 172 mg K kg^{-1}, 227 mg Mg kg^{-1}, 4 817 mg Ca kg^{-1}, sieved through 2mm meshes), (v/v) sand (Entreprises Guy Bélanger, St-Henri-Mascouche, QC: pH 7.5, <4 mg P kg^{-1}, 7 mg K kg^{-1}, 47 mg Mg kg^{-1}, 218 mg Ca kg^{-1}), and Tropical Plant Soil (Modugno-Hortibec Inc., St-Laurent, QC). The substrate was autoclaved twice for 60 min at 121°C to kill the indigenous mycorrhizal fungi. In the aim to reintroduce a microbial community exempt of mycorrhizal fungi in the growth substrate, a 500 g subsample of the sandy loam soil was mixed in 1.5 L of sterilized Milli-Q water, gently agitated for 30 min and passed through Whatman No. 1 and 42 filter papers. The filtrate was added to 5 kg of growth substrate. The resulting mix was incubated at 26°C and homogenised daily during two weeks before use. Each compartment of subunits A was supplied with a bottom layer of 20 mL quartz gravel (2 mm sieved and autoclaved for 60 min at 121°C) to favour drainage, topped with 300 mL of growth substrate, while subunits B were filled with 100 mL gravel and 2 L growth substrate.

Seeds (Société coopérative agricole du sud de Montréal, Sherrington, QC) were surface-sterilized by immersion in 70% (v/v) ethanol for 15 min, followed by 25% (v/v) commercial bleach (6% sodium hypochlorite) plus 1% (v/v) Triton X100 for 20 min and finally three rinses in sterilized distilled water. Leek seeds (*Allium porrum* L. cv. Farinto) were incubated for four days while tomato seeds (*Solanum lycopersicum*, cv. Cobra) for 48 h in darkness, at 22-25°C, in Petri dishes filled with Tryp Soy Agar (TSA, Quélab, Montreal, QC) to test the presence of contaminants. The germinated seedlings were transferred in the subunits A or B as described below. After three days, the seedlings were thinned as described before to one per compartment out of three and maintained in a greenhouse under conditions of 16h light (22°C) and 8h darkness

(20°C). The growth substrate was supplied with 20 mL Long Ashton nutrient solution five times concentrated (Hewitt, 1966) per week and watered with desionised water, as needed, to maintain the soil moist. Special care was taken not to overwater the substrate so that no liquid ever flew through holes of drainage.

Ri T-DNA-transformed carrot roots (*Daucus carota* L.) colonized with *G. intraradices* Schenck and Smith (DAOM 181 602) were grown in minimal medium solidified with 0.4% (w/v) gellan gum (Gel Gro, ICN Biochemical, Cleveland, Ohio), as described in Fortin et al. (2002). Cultures were incubated in the dark at 26°C, for six months. The spores were separated from the gel by dissolving the medium in 10 mM sodium citrate buffer (pH 6.0), at 35°C (Doner and Bécard, 1991). The spores were then suspended in sterilized distilled water and then blended 4 times, for 4 sec each time.

Spore production of *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe (BEG 12) was performed in pot cultures containing leek plants grown in 1:1:1 (w/w) sandy loam soil, sand and perlite, in a growth chamber. The soil preparation was autoclaved twice for 60 min before the inoculation of leek seedlings with root fragments colonized with *G. mosseae* from a previous culture. After verification of root mycorrhizal colonization (as described below), the spores were recovered from eight month-old pot cultures by blending the soil and roots in tap water, wet sieving through 500- and 45-µm meshes and decanting. The presence of spores in the suspension recovered from the 45-µm mesh sieve was then verified at 25× magnification, under a dissecting binocular. Further spore purification was carried out by two successive centrifugations at 3000 rpm, for 2 min, in a density gradient with a 60% (w/v) sucrose layer at the bottom. The spores were collected from the gradient interface with a syringe and thoroughly washed

with sterilized distilled water. They were then slowly vortexed in sterilized distilled water plus one drop of Tween 80 and washed abundantly in sterilized distilled water, three times successively, before being stored overnight at 4°C in sterilized distilled water. *G. mosseae* spores were surface disinfected by the use of a Buchner filtration system fitted with a Whatman No. 4 filter paper. Incubations and rinses were carried out while gently shaking suspensions with a sterilized spoon. The spores were first suspended in 70% (v/v) ethanol, for 30 sec, rinsed three times with sterilized distilled water, next immersed 20 min in 2% (v/v) chloramine T plus one drop of Tween 80, and later rinsed ten times, one min, with sterilized distilled water. They were afterwards incubated for 24 h in Tryp Soy Broth solution (Quélab) with 0.025% (w/v) ampicillin and 0.01% (w/v) streptomycin sulfate at 24°C in darkness. They were then transferred to a new Buchner filtration system, rinsed abundantly in sterilized distilled water, incubated for 20 min in 2% chloramine T and rinsed again ten times for 1 min to be finally suspended in sterilized distilled water and stored at 4°C.

The AMF spores viability was estimated by incubation in 0.1% (v/v) MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) solution for 72 h at 22°C (Walley and Germida, 1995). Two tests were performed with 1 mL spore suspension of each *Glomus* species, with three counts per test. The total number of spores colored in pink or red (considered alive) was counted at 18X magnification. Prior to inoculation, the spores were cold-treated at 4°C for 2 weeks in sterilized distilled water (Juge et al., 2002).

P. nicotianae Breda de Haan (isolate 201) was grown on autoclaved V8 agar [V8 juice (Campbell Company, Toronto, ON) diluted 1:10 in Milli-Q water, 0.2% (w/v) $CaCO_3$, 1% (w/v) gellan gum (Gel Gro), 0.005% (w/v) piramicin, 0.025% (w/v)

ampicillin, 0.001% (w/v) rifampicin] at 26°C, under conditions of 16h light and 8h darkness (Tuite, 1969). After two weeks, in order to collect the inoculum necessary for each experimental unit, the mycelium on the surface of ten Petri dishes was removed with a sterilized scalpel blade, mixed with 100 mL sterilized Milli-Q water and blended two times for 4 sec.

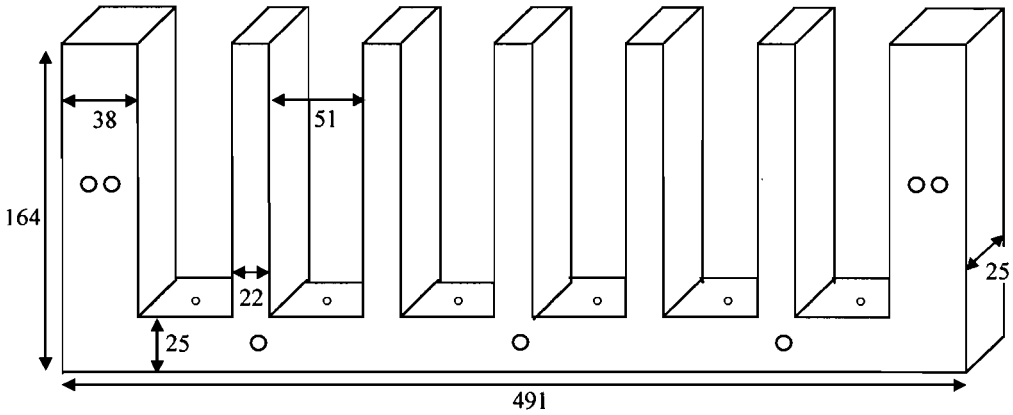
Description of the compartmentalized microcosms and experimental setting

The microscosm used in this experiment was based on the container system described by Wyss et al. (1991) with modifications. Microcosms (subunits A, B and C, Fig. 9) were built using polyethylene plates (Plastroph, St-Etienne-de-Lauzon, QC) in order to compartmentalize the soil. A 48 μm nylon membrane (Sefar America, Buffalo, NY) which permits microbial but not root growth was glued with silicone adhesive sealant (Superflex Clear RTV, Loctite, Mississauga, ON) on one side of subunits C. Subunits A and/or B (used for plant inoculation) were assembled as described below by placing subunits C in-between for separation. The microcosms were closed with full plates and supported with screws, bolts, eyelets and filing clamps.

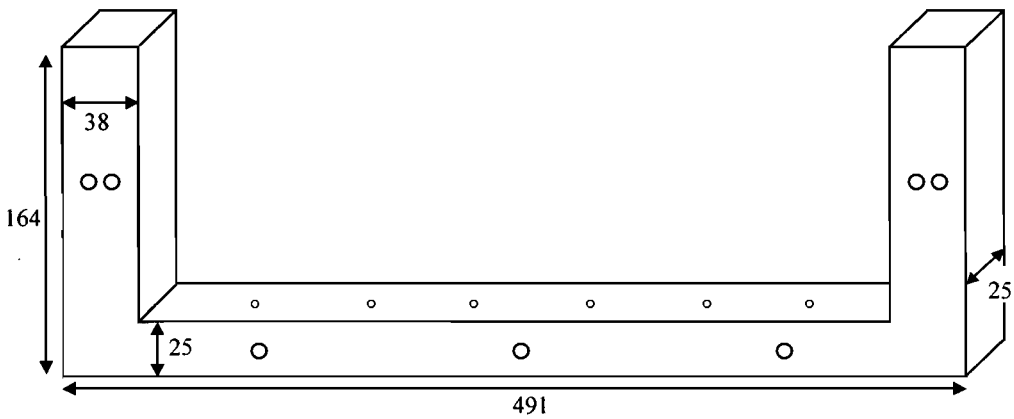
In the first step (Fig. 10), an acetate was glued on each side of subunits A by the use of silicone sealant in order to isolate every compartment. Depending on the treatment, the acetate was either cut on one side of the compartments to permit tomato plant colonization with AMF (treatment Gi or Gm) or it was left intact to block the growth of AMF towards the plants (treatment G-). The subunits A were then placed between two subunits B containing leek plants either colonized with *G. intraradices* (on one side) or with *G. mosseae* (on the other side), and tightly joined together as described previously. Tomato seedlings were transferred in the subunits A and thinned

Figure 9. Subunits used to compartmentalize the soil (side view), with dimensions indicated in mm. All subunits were made of polyethylene plates. Subunits A were composed of six compartments, for the individual growth of tomato plants whereas subunits B of only one compartment. Subunits C consisted in plates with six openings, on which a nylon membrane with 48 μm pores was glued on the side facing subunits A, to prevent root but permit microbial growth between compartments. Full plates of the same size but without openings were used to close the microcosms on both sides. Holes of 7 mm diameter were used to screw the subunits together. Holes of 1 mm diameter were drilled through the bottom for drainage.

A.



B.



C.

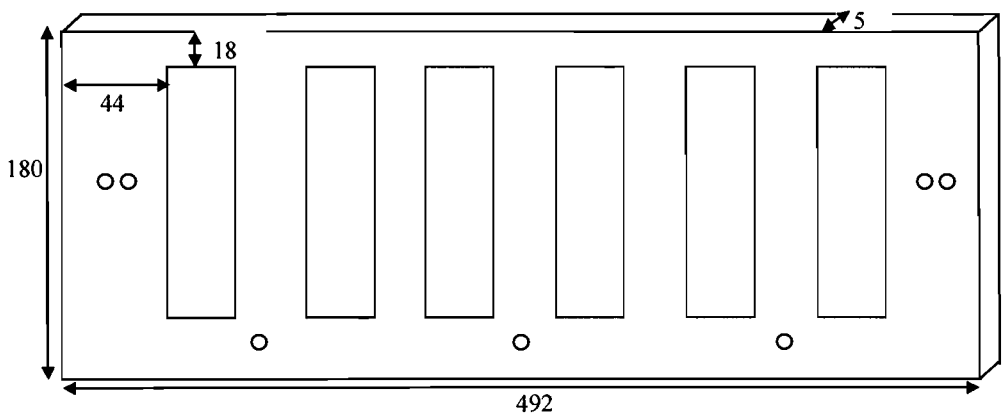
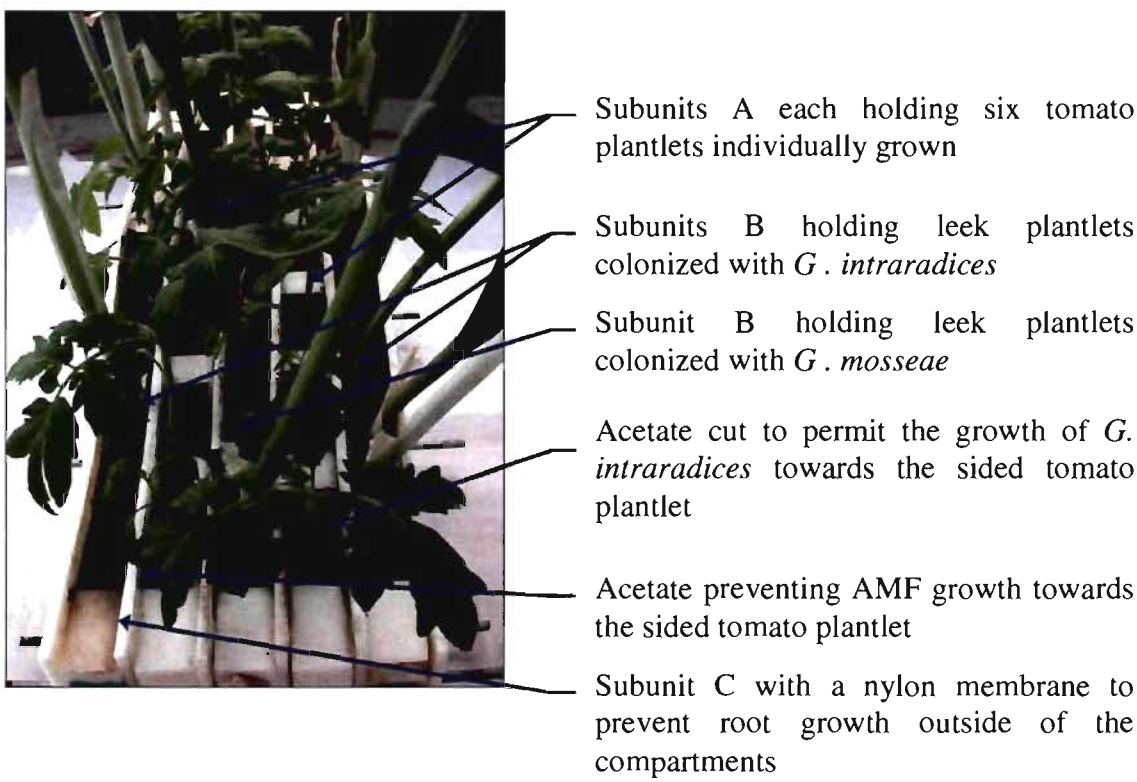


Figure 10. First step in setting the experiment. Tomato plants, individually grown in subunits A were sided to subunits B, containing leek plants colonized with *G. intraradices* or *G. mosseae*. An acetate, glued on both sides of each subunit A, was carefully cut on the side facing the appropriate compartment, to permit mycorrhizal growth when required. Separation was performed by the use of a subunit C with a nylon membrane (48 μm diameter pores) glued on one side. Full plates, screws, bolts, eyelets and filing clamps (removed here for more visibility) were used to keep the system tightly closed.



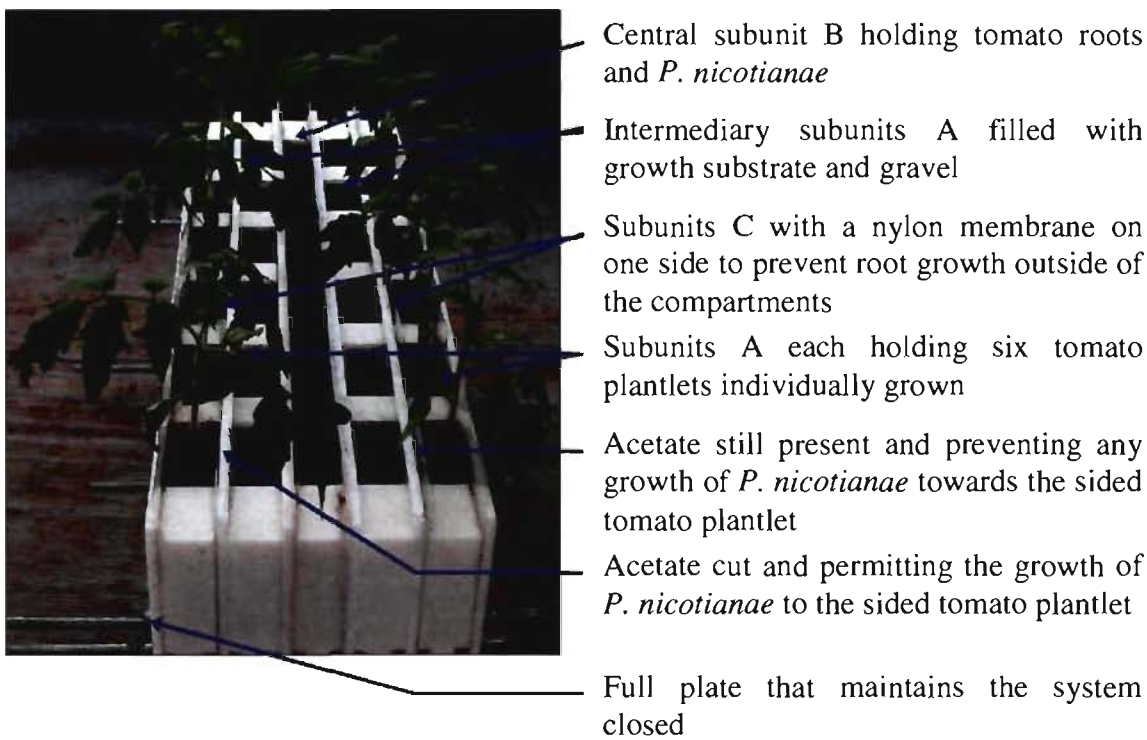
to one seedling per compartment. Two subunits A were prepared for each experimental unit. Two weeks after planting and until harvest, 2 mL of root exudates (collected from tomato plants colonized with *G. mosseae*, with *G. intraradices* or non-colonized) or sterilized Milli-Q water (Milli-Q synthesis, RiOs^{TM}, Millipore, Mississauga, ON) were applied daily on the soil of each compartment of subunits A. After three weeks from seedling inoculation, required for mycorrhizal colonization, subunits B containing leek plants were taken away and subunits A were inoculated with *P. nicotianae* as described below.

Inoculation of tomato plants

To ensure a fast and homogenous mycorrhizal colonization, tomato plants were grown in subunits A leaned to subunits B holding leek plants already colonized with either *G. intraradices* or with *G. mosseae*. In each subunit B, leek seedlings were sown and thinned to six plants. After two weeks, each subunit B was opened and a water suspension of 500 viable spores of *G. intraradices* or *G. mosseae* were poured on the sub-apical zone of each plantlet root system. After seven weeks, root colonization was verified (as described below) on a root subsample from each subunit B then ready to be used to inoculate tomato plants.

In order to prepare the central compartment used as a source of *P. nicotianae* inoculum, a 150 μm nylon membrane was glued on both sides of a subunit B, using silicone sealant. Twelve tomato seedlings were sown and grown for one week, before a 100 ml suspension of *P. nicotianae* mycelium was evenly spread on the surface of the substrate. Two weeks later, the infected tomato plants were thinned and the subunit was incorporated in the experimental system. The acetates on each side of subunits A

Figure 11. Second step of the experiment. New acetates, glued on both sides of subunits A already containing AM colonized or control plants, were carefully cut facing the appropriate compartment to permit *P. nicotianae* growth towards the sided tomato plantlet when required. The central subunit B contained thinned tomato plants infected with *P. nicotianae* and the two intermediary subunit A held gravel and growth substrate only. Separation was performed with a subunit C with a nylon membrane (48 µm pores diameter) glued on one side. Full plates, screws, bolts, eyelets and filing clamps (removed here for more better visibility) were used to close the system. Such an assemblage then presented one experimental unit, composed of twelve treatments.

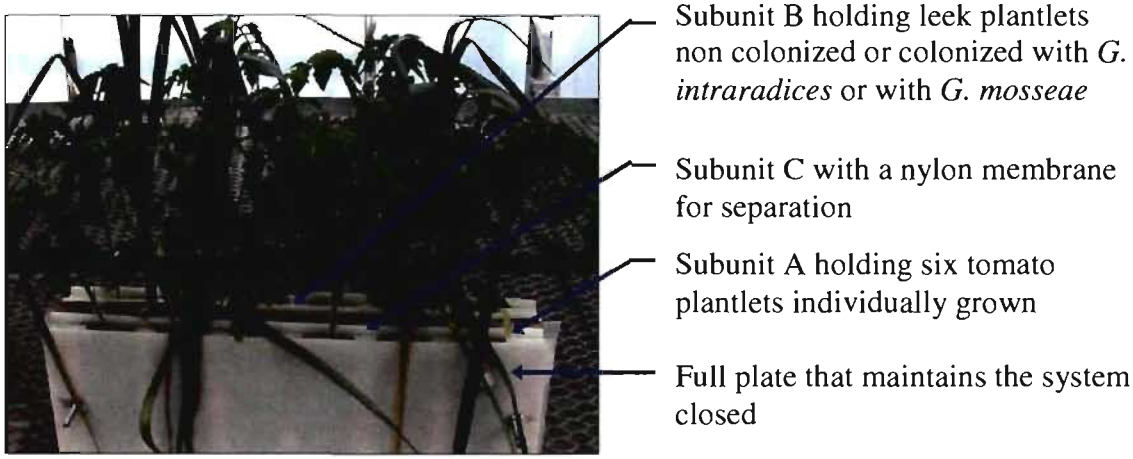


containing tomato plants previously inoculated with AMF or treated with root exudates were replaced by new ones. An opening was cut, facing the appropriate compartments, to allow the growth of *P. nicotianae* towards the tomato plants (treatment P+) or the acetate was left intact to block the pathogen growth towards the plants (treatment P-). Each subunit A containing the tomato plants was joined to a second subunit A (then called intermediary subunit) only filled with gravel and growth substrate. The subunit B inoculated with *P. nicotianae* was then set in-between the two such subunits A constructions (Fig. 11).

Production of tomato root exudates

Root exudates of tomato plants colonized with *G. intraradices*, *G. mosseae* or without mycorrhizal colonization were collected according to Pinior et al. (1999). Subunits B containing leek plants colonized with either *G. intraradices*, *G. mosseae* or non-AM inoculated were placed between two subunits A and the whole system was tightly joined together as described previously (Fig. 12). One inoculated tomato seedling was allowed to grow in each compartment of subunits A. After five weeks, the root system of each tomato plant was gently washed under tap water and incubated in Erlenmeyer flasks filled with 100 mL sterilized Milli-Q water, for 22h, in the same greenhouse as for the main experiment. Solutions were successively passed through Whatman No. 4 and No. 42 filter papers and then 0.22 µm nitrocellulose filters (Millipore). The root exudates were concentrated to a ratio of 1 g of root fresh weight equivalent to 20 mL of exudate solution by lyophilization. The pH of the solutions (and of sterilized Milli-Q water used as a control) was then adjusted to 6.0 before sterilization by filtration through 0.22 µm nitrocellulose filters and storage at -20°C

Figure 12. Experimental setup used to produce tomato root exudates. The central subunit B contained leek plants non-colonized or colonized with *G. intraradices* or *G. mosseae*. On each side, one subunit A contained six tomato plants, grown in individual compartments. Separation was performed with a subunit C with a nylon membrane (48 µm diameter pores) glued on one side. Full plates, screws, bolts, eyelets and filing clamps (removed here for better visibility) were used to close the systems.



until use. The extent of mycorrhizal root colonization of the plants was later assessed as described below.

Plant harvest and measurements

After six weeks of growth, the totality of the substrate was withdrawn from the compartments containing tomato plants. The shoot dry weight was determined after drying at 80°C for 72h. The total root system was separated from the soil using sterilized forceps and gloves. The soil was kept at -20°C until use for DGGE analysis (cf. chapter VI). The percentage of root apexes presenting necrosis was estimated at 18× magnification, under a dissecting binocular. The root fresh weight was then determined. The roots were later cut in 1 cm sections and mixed in sterilized distilled water. A first subsample was used for quantification of *P. nicotianae* in tomato root extracts, using a commercial ELISA kit (*Phytophthora* Pathoscreen kit, Agdia, Elkhart, IN) and following the suppliers instructions. A second subsample was used to assess mycorrhizal colonization after the roots were cleared in 10% (w/v) KOH for 5 min at 90°C, abundantly rinsed under tap water and stained for 5 min in 5% (v/v) black ink (Shaeffer, Madison, IA) in 5% (v/v) acetic acid solution at 90°C (Vierheilig et al., 1998). The roots were finally rinsed and suspended in tap water before the percentage of root length colonized by AMF was estimated using the gridline intersect method (Giovannetti and Mosse, 1980).

Statistical analyses

The data analyses were performed using General Linear Model Procedures of SAS/STAT software, version 9.1.3 of the SAS system for Windows (SAS Institute Inc. 2004, Cary, N.C.). The effects of AM fungi inoculation (E-G-, E-Gi, E-Gm), exudate application ($E^{G-}G-$, $E^{Gi}G-$, $E^{Gm}G-$) and the inoculation of *P. nicotianae* (P+, P-) on the level of root necrosis, root mycorrhizal colonization, shoot dry weight, root fresh weight and *P. nicotianae* proliferation within tomato roots (quantified by optical density values following ELISA tests) were analyzed by ANOVA. The statistical model used included blocks, treatments and the appropriate interactions. A posteriori comparisons were performed by LSD tests (concerning exudates application and mycorrhizal inoculation treatments) or Tukey studentized range tests (concerning *Phytophthora* inoculation treatment). Rank transformation of the optical density data was performed before the analysis in order to meet ANOVA requirements.

Results

Root colonization with *G. mosseae* or *G. intraradices* significantly reduced *P. nicotianae* proliferation in roots ($P<0.05$), as measured by ELISA tests, in comparison to non-colonized roots (Table V). Application of root exudates collected from plants colonized with the same AM fungal species had no significant effect on *P. nicotianae* biomass in roots, in comparison to control plants supplied with exudates from non-mycorrhizal plants or sterilized pure water. There was no significant interaction between treatments and *P. nicotianae* was not detected in control plants non-inoculated with the pathogen.

Mycorrhizal colonization of tomato root was slightly higher ($P=0.08$) in plants inoculated with *G. mosseae* than with *G. intraradices,* with respectively 45.0% and 23.4% of root length bearing mycorrhizal fungi structures. No mycorrhizal colonization was detected in control plants not inoculated with the AMF. Inoculation of *P. nicotianae* did not alter mycorrhizal colonization and there was no significant interaction between AMF and *P. nicotianae* inoculation treatments (Table V).

There was a significant increase of the shoot dry weight ($P<0.01$; Fig. 13) and the root fresh weight ($P<0.05$; Fig. 14) in tomato plants infected with *P. nicotianae* growing from the inoculated compartment. On the other hand, inoculation with *G. intraradices* or *G. mosseae* as well as the application of exudates from mycorrhizal or non-mycorrhizal plants did not modify the shoot dry weight nor the root fresh weight compared to control plants non inoculated with AMF and filled with pure water. There was no interaction between pathogen inoculation and the combination of mycorrhizal inoculation and exudate application treatments.

The percentage of root length showing necrosis was not significantly modified by *P. nicotianae* infection, mycorrhizal colonization and root exudate application (P=0.56) and there was no interaction between treatments (Fig. 15).

Table V. Effect of mycorrhizal inoculation and exudate application on mycorrhizal colonization and on *Phytophthora* proliferation in tomato roots, after six weeks of growth

Exudate application[1]	**AMF inoculation**[2]	**Pathogen inoculation**[3]	**Mycorrhizal colonization (%)**[4]	***Phytophthora* OD**[5]
Control	Non myc	Control	0.0	0.02 b [6]
Control	*G. intra*	Control	22.4	0.00 b
Control	*G. moss*	Control	47.9	0.00 b
Control	Non myc	*P. nicot*	0.0	0.33 a
Control	*G. intra*	*P. nicot*	24.5	0.04 b
Control	*G. moss*	*P. nicot*	41.9	0.00 b
Exu non myc	Non myc	Control	0.0	0.00 b
Exu *G. intra*	Non myc	Control	0.0	0.03 b
Exu *G. moss*	Non myc	Control	0.0	0.00 b
Exu non myc	Non myc	*P. nicot*	0.0	0.32 a
Exu *G. intra*	Non myc	*P. nicot*	0.0	0.27 a
Exu *G. moss*	Non myc	*P. nicot*	0.0	0.59 a

[1] Control : application of Milli-Q water; Exu non myc: exudates collected from non colonized plants; Exu *G. intra*: exudates collected from plants colonized with *G. intraradices*; Exu *G. moss*: exudates collected from plants colonized with *G. mosseae*.

[2] Non myc : plants non AMF inoculated; *G. intra*: plants inoculated with *G. intraradices*; *G. moss*: plants inoculated with *G. mosseae*.

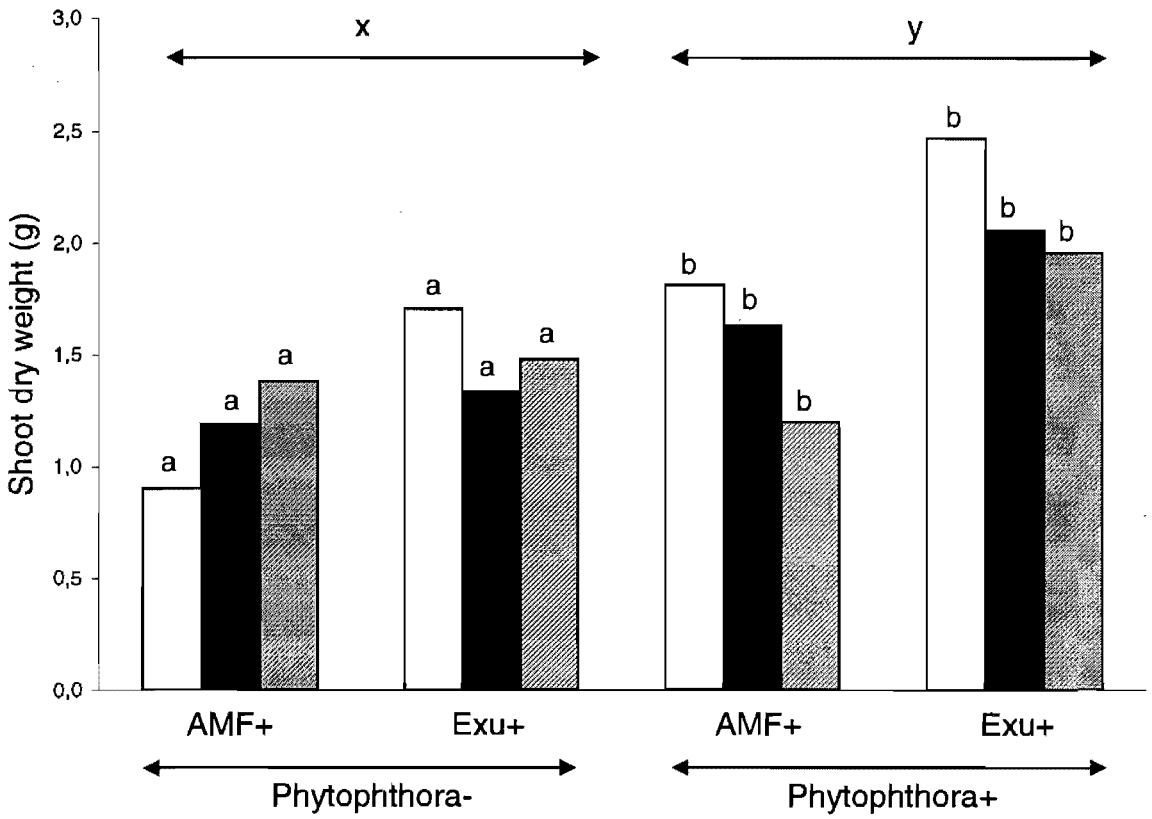
[3] Control: plants not inoculated with *P. nicotianae*; *P. nicot*: plants inoculated with *P. nicotianae*.

[4] Percentage of root length bearing mycorrhizal colonization.

[5] *Phytophthora* proliferation quantified by ELISA tests, expressed in absorbance units.

[6] Values with different letters were significantly different at $P<0.05$.

Figure 13. Shoot dry weight of tomato plants after six weeks of growth in a compartmentalized system.

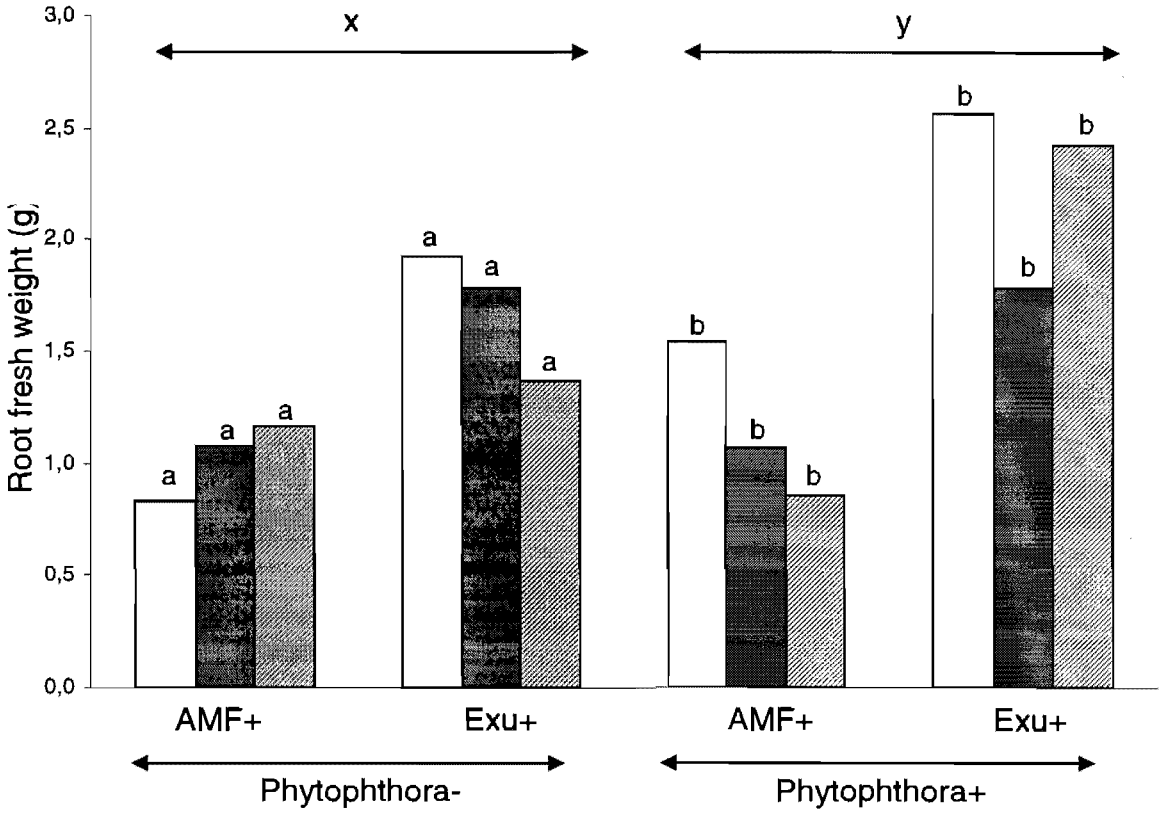


Phytophthora-: the system prevented *P. nicotianae* growth towards the tomato roots.
Phytophthora+: the system permitted *P. nicotianae* growth towards the tomato roots.
AMF+: half of the plants were either inoculated with *G. intraradices* (■), *G. mosseae* (▨) or non inoculated (□) and supplied with water as a control for exudate application.
Exu+: the other half of the plants were non-AMF inoculated and filled with exudates from non mycorrhizal plants (□), or from plants colonized with *G. intraradices* (■) or *G. mosseae* (▨).

Overall, there was a significant increase ($P<0.005$) of shoot dry weight (x, y) in *P. nicotianae* inoculated plants.

Within plants non-inoculated (a) or inoculated (b) with *P. nicotianae*, a same letter denotes no significant difference between exudates application and AMF inoculation treatments.

Figure 14. Root fresh weight of tomato plants after six weeks of growth in a compartmentalized system.

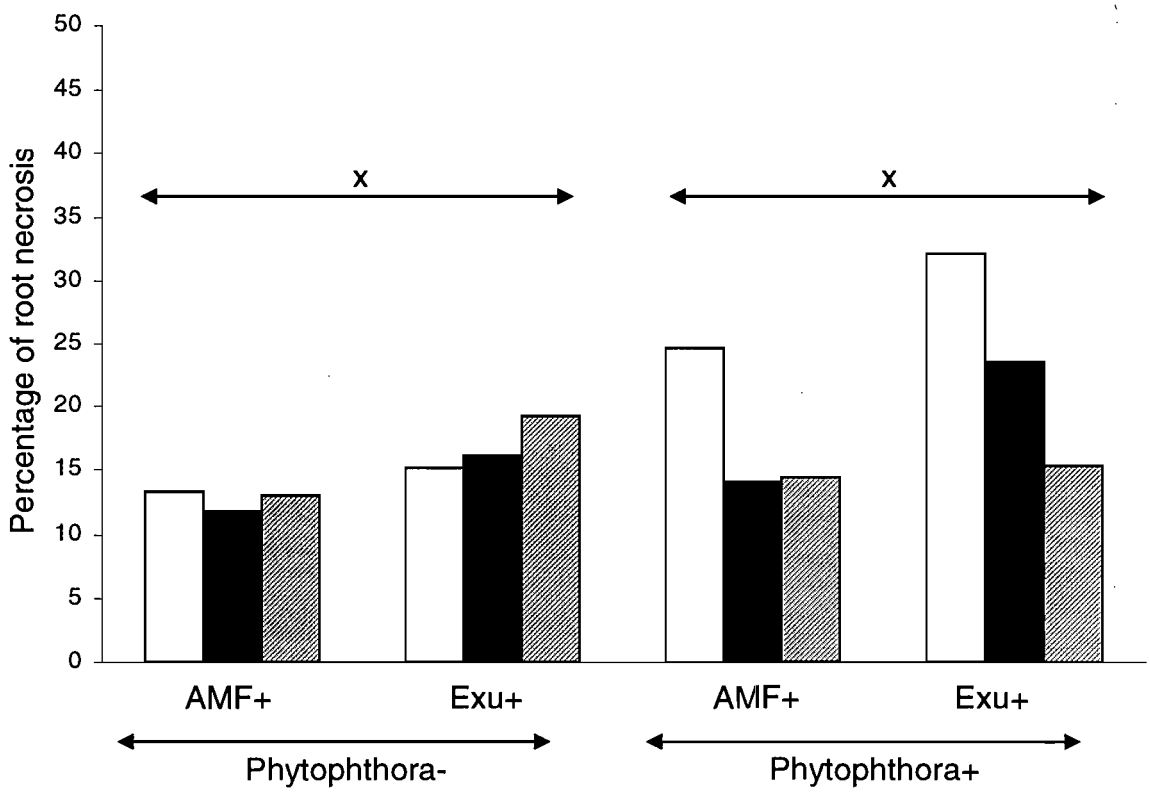


Phytophthora-: the system prevented *P. nicotianae* growth towards the tomato roots.
Phytophthora+: the system permitted *P. nicotianae* growth towards the tomato roots.
AMF+: half of the plants were either inoculated with *G. intraradices* (■), *G. mosseae* (▨) or non inoculated (□) and supplied with water as a control for exudate application.
Exu+: the other half of the plants were non-AMF inoculated and filled with exudates from non mycorrhizal plants (□), or from plants colonized with *G. intraradices* (■) or *G. mosseae* ( ).
▨

Overall, there was a significant increase ($P<0.005$) of root fresh weight (x, y) in *P. nicotianae* inoculated plants.

Within plants non-inoculated (a) or inoculated (b) with *P. nicotianae*, a same letter denotes no significant difference between exudates application and AMF inoculation treatments.

Figure 15. Percentage of root necrosis of tomato plants after six weeks of growth in a compartmentalized system.



Phytophthora-: the system prevented *P. nicotianae* growth towards the tomato roots.
Phytophthora+: the system permitted *P. nicotianae* growth towards the tomato roots.
AMF+: half of the plants were either inoculated with *G. intraradices* (■), *G. mosseae* (▨) or non inoculated (□) and supplied with water as a control for exudate application.
Exu+: the other half of the plants were non-AMF inoculated and filled with exudates from non mycorrhizal plants (□), or from plants colonized with *G. intraradices* (■) or *G. mosseae* (▨).

Overall, there was no effect of *P. nicotianae* inoculation ($P>0.15$) on the percentage of root necrosis.

Within plants non-inoculated or inoculated with *P. nicotianae*, no significant difference between exudates application and AMF inoculation treatments was denoted.

Discussion

G. mosseae but also *G. intraradices* induce the biocontrol of *P. nicotianae* infecting tomato plants

In this experiment, root colonization with *G. mosseae* BEG 12 or *G. intraradices* DAOM 181 602 reduced *P. nicotianae* infection within tomato roots, while the application of root exudates from plants colonized with the same AMF species had no effect on the pathogen. Biocontrol by *G. mosseae* BEG 12 was previously described (Cordier et al., 1996; Pozo et al., 2002a; Trotta et al., 1996; Vigo et al., 2000) whereas as far as we know, this is the first time biocontrol induced by *G. intraradices* is observed on tomato plants challenged with this pathogen. The lack of effect of root exudates from mycorrhizal plants on *P. nicotianae* infection suggests that the biocontrol induced by *G. mosseae* and *G. intraradices* is not mediated by a modification in root exudation after root colonization with AMF.

P. nicotianae did not induce disease symptoms

Under our experimental conditions, inoculation of *P. nicotianae* in a central system did not increase tomato root necrosis. The intraradical proliferation of the pathogen was probably not sufficient to induce the apparition visible symptoms. Nonetheless, it was detected by ELISA tests and was thus able to grow from the compartment of inoculation towards tomato roots and successfully infect plants. Inoculation of *P. nicotianae* from the central subunit significantly increased the tomato plants shoot dry weight and root fresh weight, probably because of the availability of additional nutrients from the organic matter of the substrate of central and intermediary

subunits, as well as from the degradation of the infected tomato roots used to support the pathogen growth within the central subunit. These additional nutrients available o the tomato plantlets may have compensated for the deleterious effect of the pathogen on shoot dry weight and root fresh weight.

Exudates from mycorrhizal plants did not reduce the intraradical growth of *P. nicotianae*

Using a biotest, *in vitro*, we showed an increased attraction of *P. nicotianae* zoospores by exudates collected from 16 week-old transformed tomato roots colonized with the same isolate of *G. intraradices* as in this study, but observed a lower attraction (and even repulsion) by exudates from 24 week-old roots (cf. chapter IV). These results suggested the possible liberation by tomato roots of attractive and repulsive molecules to *P. nicotianae* zoospores with concentrations changing with root and mycorrhizal ageing. In this study, inoculation of *P. nicotianae* consisted in mycelium supplied on tomato roots then leaned to the studied plants. From the inoculated roots, the pathogen would have developed mycelium but also zoospores (Erwin and Ribeiro, 1999). A positive chemotropism of germ tubes towards root exudates has also been shown (Carlile, 1983; Deacon and Donaldson, 1993; Zentmyer, 1970). Nonetheless, the chemotactic responses of this inoculum, within soil, may be different in comparison to the one adopted by zoospores *in vitro*. Moreover exudates were collected after five weeks only after sowing. It is possible that they did not present repulsion at that harvest time. Using a similar exudate concentration as we used here, Pinior et al. (1999) showed that cucumber root exudates from plants colonized with *G. mosseae* inhibited a further root colonization with this AMF, in comparison to water-treated controls. This

effect was later shown to be systemic as exudates (still used at the same concentration) collected from non-mycorrhizal roots of mycorrhizal plants induced similar results (Vierheilig et al., 2003). If similar molecules are involved in the inhibition of *P. nicotianae* chemotaxy *in vitro*, the absence of effects we noticed here would therefore probably not be related to an inadequate exudate concentration.

Exudates from mycorrhizal plants may impact the reproductive but not the vegetative development of pathogens

Exudates from mycorrhizal plants or mycorrhizal structures were also tested on the formation or the germination of propagules of various pathogens by other authors. Exudates from non mycorrhizal strawberry plants stimulated more the sporulation of *P. fragariae in vitro* than exudates from plants colonized with *G. etunicatum* or *G. monosporum* (Norman and Hooker, 2000). The authors observed a similar pattern when the pathogen was inoculated in the mycorrhizosphere of strawberry plants colonized with *G. etunicatum*. Microconidia germination of the pathogen *Fusarium oxysporum* f. sp. *lycopersici* was more than doubled in the presence of root exudates from tomato plants colonized with *G. mosseae* in comparison to exudates from non mycorrhizal plants. The more the tomato roots were colonized by the AMF, the more microconidia germination was increased (Scheffknecht et al., 2006). Similar results were obtained with exudates from non-hosts plants of the same and other plant family showing that the changes in root exudation affecting *Fol* microconidia germination do not occur exclusively in tomato and thus are non-specific (Scheffknecht et al., 2007). Moreover, extracts from *G. intraradices* grown in a root free compartment in comparison to controls free of AMF were shown *in vitro* to stimulate both the growth of *Pseudomonas*

chlororaphis and of *Trichoderma harzianum*, while a reduced conidial germination of *F. o.* f. sp. *chrysanthemi* and no effect on the growth of *Clavibacter michiganensis* were observed (Filion et al., 1999). This is the first time exudates collected from mycorrhizal plants were directly applied to plants challenged with a pathogen. Root exudates from mycorrhizal plants may impact the formation, the germination and/or the attraction of pathogen propagules within the soil, but this would not influence their vegetative development within roots after infection. The reduction of a pathogen ability to get in contact with roots after their colonization with AMF would not impact its capacity to invade and proliferate within host tissues. Application of the fungicide carbendazim decreased the level of AMF colonization and increased the percent of root length infected with oospores of *Aphanomyces euteiches* (applied to the soil by adding oospores produced *in vitro*), under field conditions (Bødker et al., 2002). No correlation was found between AMF colonization and disease severity, disease incidence or pathogen enzymatic activity (glucose-6-phosphate dehydrogenase). The authors suggested that AMF would not influence the vegetative stage of pathogen development during which root rotting takes disease but rather the reproductive stage when oospores are produced.

The chemotactic responses of the plant-growth–promoting rhizobacteria *Azotobacter chroococcum* and *Pseudomonas fluorescens* to exudates of tomato plants colonized with *G. fasciculatum* were significantly stronger than the response to exudates of non-AM roots, and was shown to increase with an augmentation in exudate concentration (Sood, 2003). These bacteria were also significantly more attracted by tomato roots colonized by this AMF than non mycorrhizal roots. A change in the attraction of rhizobacteria by root exudates after mycorrhizal colonization, if it also

happened in the present experiment, would not impact the proliferation of the pathogen within host roots.

Possible mechanisms of the biocontrol mediated by AMF

AMF are able to directly impact the mycorrhizosphere microflora by mechanisms that have still to be described (Andrade et al., 1997; Mansfeld-Giese et al., 2002; Marschner and Timonen, 2005; Marschner et al., 1997; 2001; Söderberg et al., 2002; Wamberg et al., 2003). This microflora would then be unfavourable to a further pathogen proliferation within the mycorrhizosphere and then within the host roots. *Bacillus simplex*, *Bacillus* sp. and *Paenibacillus* spp. identified associated with surface disinfected spores of *G. mosseae* exerted various levels of antagonism *in vitro* over *P. nicotianae*, *F. solani,* and three isolates of *F. oxysporum* (cf. chapter VII). *Paenibacillus* sp. strain B2 also isolated from the mycorrhizosphere of *G. mosseae* reduced tomato root necrosis caused by *P. nicotianae*, displayed cellulolytic, proteolytic, chitinolytic and pectinolytic activities and were shown to liberate polymixin B1 and two other polymyxin-like compounds antagonistic among *F. solani* and *F. acuminatum* (Budi et al., 1999; 2000; Selim et al., 2005). These endobacteria may directly impact a pathogen within the mycorrhizosphere and may contribute to the biocontrol mediated by AMF on soilborne diseases.

The synthesis of constitutively expressed β-1,3-glucanase was increased and additional and specific isoforms of chitinase and β-1,3-glucanase were detected in roots colonized with *G. mosseae* (Pozo et al., 1996; 1998; 1999). These modifications were not observed in the non-mycorrhizal part of a *G. mosseae* mycorrhizal plant grown in a split-root system, even if these roots presented a reduced proliferation of the pathogen,

a higher lytic activity against this pathogen and less damage in comparison to non-mycorrhizal plants (Pozo et al., 2002a). Cordier et al. (1998) postulated that *G. mosseae* colonization leads to induced systemic resistance and identified localized cell-wall modifications such as callose accumulation around arbuscule-containing cortical cells. They also noted the accumulation of callose in the form of papillae in root-cells invaded by the pathogen and the accumulation of PR-1 proteins characteristic to systemic resistance in tomato roots colonized with *G. mosseae,* that were not noticed without pathogen inoculation. In a split-root system, they observed the reduction of *P. nicotianae* development and root damage not only in the mycorrhizal part but also in the non-mycorrhizal part of the tomato roots. Stimulation of plant defense mechanisms would therefore play an important role in biocontrol by *G. mosseae* on tomato plants infested by *P. nicotianae*. The induction of such mechanisms induced by *G. intraradices* will have to be tested. Nonetheless, the accumulation of JA implicated in plant disease resistance and in the rhizobacteria-mediated induced systemic resistance (JA) (Pozo et al., 2004) in roots colonized with both AMF [precisely within arbuscule-containing cells (Hause et al., 2007; Hause et al., 2002; Isayenkov et al., 2005)], could be related to pathogen biocontrol.

Other mechanisms such as a direct competition for space and nutrients between pathogens and AMF within the soil and the host roots may also be involved in the biocontrol we observed in this study. Larsen and Bødker (2001), using signature fatty acids profiles, demonstrated the decrease in biomass and energy reserves of both *G. mosseae* and *A. euteiches* co-occupying pea roots. Cordier et al (1996) also showed that *P. nicotianae* and *G. mosseae* never occupied simultaneously the same tomato root tissues. A reduction in the extent of mycorrhizal colonization by different plant

pathogens has been reported (Bååth and Hayman, 1983; Davis and Menge, 1980; Krishna and Bagyaraj, 1983), indicating the possible occurrence of competitive interactions.

Conclusion

In this experiment, root exudation modification after colonization with *G. mosseae* and *G. intraradices* did not impact the proliferation of *P. nicotianae* within tomato roots. Thus, the biocontrol induced by AMF may not be determined by a change in pathogen chemotactic response to the host root exudates but by other mechanisms that still have to be precised and may occur differently depending on the combination of plant-pathogen-AM species and environmental conditions.

Acknowledgments

We are grateful to Franck Panabières (INRA Antibes) for providing the *P. nicotianae* isolate 201, to Robert Mowatt (Plastroph, St-Etienne-de-Lauzon, QC) for the microcosms construction, to Stéphane Daigle for performing the statistical analysis and to the undergraduate student Anne Keough for excellent technical assistance. This research was supported by an NSERC grant allocated to M. St-Arnaud and an FQRNT grant to M. Jolicoeur.

Chapitre VI

The impact of arbuscular mycorrhizal fungi on the bacterial community structure of tomato rhizosphere would not be related to root exudation modification and would not be affected by inoculation with Phytophthora nicotianae

Laëtitia Lioussanne, Mario Jolicoeur & Marc St-Arnaud

Les résultats de ce chapitre ont été présentés lors des congrès indiqués ci-bas et seront prochainement soumis afin de publication dans une revue internationale.

Lioussanne, L., M. Jolicoeur et M. St-Arnaud. 2006. The inhibition of *Phytophthora nicotianae* induced by AMF is not related to tomato root exudation transformation. *Fifth International Conference on Mycorrhiza*, Granada, Spain, pp. 249.

Lioussanne, L., M. Jolicoeur et M. St-Arnaud. 2006. Is the inhibition of *Phytophthora nicotianae* in tomato roots induced by arbuscular mycorrhizal fungi related to changes in root exudation? 2006 *Joint Meeting of The American Phytopathological Society - The Canadian Phytopathological Society - Mycological Society of America*, July 29– August 2, 2006, Québec, QC, pp. 175. Can. J. Plant Pathol. 28: 357.

Abstract

Arbuscular mycorrhizal fungi (AMF) have been shown to induce the biocontrol of soilborne diseases, to change the composition of root exudates and to modify the bacterial community structure of the rhizosphere, leading to the formation of the mycorrhizosphere. We first verified if tomato colonization with the AMF *Glomus mosseae* or *G. intraradices* significantly modified the rhizosphere bacterial community. We secondly tested if the notified changes occured through root exudation modification. We lastly assessed if these modifications were influenced by inoculation with the soilborne pathogen *Phytophthora nicotianae*. Tomato plants were grown in a compartmentalized soil system and were either submitted to mycorrhizal colonization or to application of exudates from mycorrhizal tomato plants, with the corresponding negative controls. Three weeks after planting, the plants were inoculated (or not, as a control) with *P. nicotianae* growing from a compartment inoculated with this pathogen and placed at an equal distance to every plant. At harvest, a PCR-DGGE analysis of a 16S rRNA gene fragment amplified from the DNA extracted from the rhizosphere was performed. Root colonization with *G. intraradices* or *G. mosseae* induced significant changes in the bacterial community structure. Conversely, the bacterial community structure was similar in rhizosphere supplied with exudates collected from mycorrhizal and from non-mycorrhizal roots. Therefore, our results suggest that AMF would modify the bacterial community structure of the rhizosphere through mechanisms unrelated to modification of root exudation. Moreover, infection with *P. nicotianae,* of mycorrhizal or non-mycorrhizal plants, did not significantly affect the rhizosphere bacterial

community suggesting that it would be weakly sensitive to pathogen invasion. Results are discussed in the aim to explain the biocontrol mediated by AMF.

Keywords

Mycorrhizosphere, arbuscular mycorrhizal fungi, *Phytophthora nicotianae*, bacterial community, exudates, DGGE

Introduction

Arbuscular mycorrhizal fungi (AMF) have been shown to reduce disease development in a wide number of plant-pathogen associations (St-Arnaud and Vujanovic, 2007). Reduction of the detrimental effects of the soilborne pathogen *Phytophthora nicotianae* on tomato plants colonized by the AMF *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe (Cordier et al., 1996; Pozo et al., 2002a; Trotta et al., 1996; Vigo et al., 2000) but also by *G. intraradices* Schenck and Smith (DAOM 181 602) (cf. chapter V) has been described. Lioussanne et al. (cf. chapter IV) showed *in vitro* the repelling of zoospores of this soilborne pathogen by exudates collected from mature transformed tomato roots colonized with *G. intraradices*. However, the application of exudates from tomato roots colonized with *G. intraradices* or *G. mosseae* on tomato plants grown in soil failed to reduce the intraradical growth of the pathogen (cf. chapter V), suggesting that other mechanisms would be involved in the AMF-mediated biocontrol.

AMF impact the other soil microorganisms development leading in this manner to the formation of the specific zone of soil called the mycorrhizosphere (Linderman, 1988). Root colonization with AMF has most of the time been shown to decrease (Bansal and Mukerji 1994; Cavagnaro et al., 2006; Christensen and Jakobsen, 1993), but also to increase (Albertsen et al., 2006; Bagyaraj and Menge, 1978; Meyer and Linderman, 1986a; Posta et al., 1994) and even to have no effect (Olsson et al., 1996b) on the microbial biomass within not only the rhizosphere (the zone of soil influenced by roots) but also within the mycosphere (the zone of soil only influenced by the mycorrhizal mycelium). AMF also have species-specific impacts on the rhizosphere by

stimulating or inhibiting the growth of microorganisms, depending on their taxon (Ames et al., 1984; Andrade et al., 1997; Corgié et al., 2006; Vazquez et al., 2000). Furthermore, some rhizobacteria have been shown to reduce pathogen proliferation especially through direct liberation of toxic compounds, competition for space and nutrients, reduction of Fe and Mn availability, modification of the plant hormone balance and stimulation of plant defense mechanisms (Azcón-Aguilar and Barea, 1996; Bowen and Rovira, 1999; Nehl et al., 1997; Vassilev et al., 2006). AMF may induce the formation of a bacterial community unfavourable to the development of pathogen within the mycorrhizosphere leading to the apparition of biocontrol.

AMF have been shown to quantitatively decrease the amount of root exudates but also to induce some qualitative modifications of root exudate composition (Azaizeh et al., 1995; Bansal and Mukerji 1994; Graham et al., 1981; Marschner et al., 1997; Sood, 2003). Sugars (Hooker et al.,2007) but also citric acid (Tawaraya et al., 2006) were detected in the soil solution collected from the hyphal compartment of mycorrhizal plants as mycorrhizal exudates. In this manner, many authors suggested that the decrease of soil microbial biomass and the modification of the soil microbial community induced by mycorrhizal colonization would be due to quantitative and qualitative changes of root exudates (Bansal and Mukerji 1994; Marschner et al., 1997). Filion et al. (1999) observed *in vitro* that extracts from *G. intraradices* grown in a root free compartment had differential effects on soil microbes, stimulating the growth of *Pseudomonas chlororaphis* and of *Trichoderma harzianum*, reducing conidial germination of the pathogen *Fusarium oxysporum* f. sp. *chrysanthemi* and having no effect on the growth of *Clavibacter michiganensis*. In addition, the chemotactic responses of the plant-growth–promoting rhizobacteria *Azotobacter chroococcum* and

Pseudomonas fluorescens to exudates of tomato plants colonized with *G. fasciculatum* were significantly stronger than the response to exudates of non-AM roots (Sood, 2003).

It has been estimated that from 0.1% to 10% of the microorganisms found on typical agricultural soils would be culturable using current culture media formulations (Head et al., 1998; Hill et al., 2000; Theron and Cloete, 2000; Torsvik et al., 1990). Conversely, culture independent methods based on 16S rRNA gene amplification permit the detection and the analysis of over 90% of the microorganisms that can be observed microscopically *in situ* (Hill et al., 2000). Denaturent gradient gel electrophoresis (DGGE) is a method by which fragments of DNA of the same length but with different sequences can be resolved electrophoretically. PCR products on gradient gels can be later sequenced and the results used to infer about the microbial diversity of the original sample (Head et al., 1998; Muyzer, 1999; Muyzer and Smalla, 1998; Theron and Cloete, 2000). By PCR-DGGE, it was confirmed that the microbial community within different plant rhizosphere was changed after mycorrhizal colonization (Marschner and Timonen, 2005; Marschner et al., 2001; Wamberg et al., 2003).

The aim of this study was first to test if changes of the rhizosphere microbial community induced by AMF happen through root exudation modification. We secondly assessed if the bacterial community structure of the mycorrhizosphere and/or of the rhizosphere (supplied or not with exudates from mycorrhizal roots) are modified by the inoculation of *P. nicotianae*. In this order, tomato plants were grown individually in a compartmentalized soil microcosm and submitted to mycorrhizal colonization or supplied with exudates from mycorrhizal plants, with the negative corresponding

controls. Plants were then placed at an equal distance to a compartment inoculated with *P. nicotianae*. Two AMF species were studied : *G. intraradices* Schenck and Smith and *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe. At harvest, the bacterial community structure was characterized by PCR-DGGE analysis of the 16S rRNA gene amplified from DNA directly extracted from the rhizosphere.

Materials and methods

Experimental design

Using the compartmentalized system described below, twelve tomato plants were, in a first step, either submitted to application of exudates from mycorrhizal tomato plants or to direct root colonization with AMF, with the corresponding negative controls. Thus, half of the plants was supplied with sterilised water (E-) and colonized with *G. intraradices* (Gi), with *G. mosseae* (Gm) or not colonized with an AMF (G-), as a control. The other half of the plants was soaked with tomato root exudates collected from plants colonized with *G. intraradices* (E^{Gi}), *G. mosseae* (E^{Gm}) or not colonized (E^{G-}), as a control. Three weeks later, in a second step, plants were either inoculated (P+) or non inoculated (P-) with *P. nicotianae*. Each experimental unit thus included the twelve following treatment combinations: E^{G-}G-P-, E^{Gi}G-P-, E^{Gm}G-P-, E-G-P-, E-GiP-, E-GmP-, E^{G-}G-P+, E^{Gi}G-P+, E^{Gm}G-P+, E-G-P+, E-GiP+, E-GmP+. The six mycorrhizal inoculation/exudate application treatments were randomized in the main plots, and *P. nicotianae* inoculation treatments were randomized in the subplots. The experiment included two blocks that each contained two replicates per treatment. The second block was started three weeks after the first one.

Biological material and growth conditions

The growth substrate was prepared according to chapter V. It was composed of a mixture of 2:2:1 (v/v) sandy loam soil, sand and Tropical Plant Soil (Modugno-Hortibec Inc., St-Laurent, QC). The substrate was autoclaved twice for 60 min at 121°C to kill the indigenous mycorrhizal fungi, and a microbial community exempt of

mycorrhizal fungi was then reintroduced. Each compartment of subunits A was supplied with a bottom layer of 20 mL quartz gravel (2 mm sieved and autoclaved for 60 min at 121°C) to favour drainage, topped with 300 mL of growth substrate, while subunits B were filled with 100 mL gravel and 2 L growth substrate.

Seeds (Société coopérative agricole du sud de Montréal, Sherrington, QC) were surface-sterilized by immersion in 70% (v/v) ethanol for 15 min, followed by 25% (v/v) commercial bleach (6% sodium hypochlorite) plus 1% (v/v) Triton X100 for 20 min and finally three rinses in sterilized distilled water. Leek seeds (*Allium porrum* L. cv. Farinto) were incubated for four days while tomato seeds (*Solanum lycopersicum*, cv. Cobra) for 48 h in darkness, at 22-25 °C, in Petri dishes filled with Tryptic Soy Agar (TSA, Quélab, Montreal, QC) to test the presence of contaminants. The germinated seedlings were transferred in the subunits and maintained in a greenhouse under conditions of 16h light (22°C) and 8h darkness (20°C). The growth substrate was supplied with 20 mL Long Ashton nutrient solution five times concentrated (Hewitt, 1966) per week and watered with desionised water, as needed, to maintain the soil moist. Special care was taken not to overwater the substrate so that no liquid ever flew through holes of drainage.

Spores of *G. intraradices* Schenck and Smith (DAOM 181 602) were separated from the gel after *in vitro* culture on Ri T-DNA-transformed carrot roots (*Daucus carota* L.) as described in chapter V. *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe (BEG 12) spore production was performed in pot cultures on leek plants and spore surface disinfection was carried out according to chapter V as well. The AMF spores viability was estimated by incubation in 0.1% (v/v) MTT (3-[4,5-dmethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) (Walley and Germida, 1995). Prior to

inoculation, the spores were cold-treated at 4°C for two weeks in sterilized distilled water (Juge et al., 2002).

P. nicotianae Breda de Haan (isolate 201) was grown on autoclaved V8 agar [V8 juice (Campbell Company, Toronto, ON) diluted 1:10 in Milli-Q water, 0.2% (w/v) $CaCO_3$, 1% (w/v) gellan gum (Gel Gro), 0.005% (w/v) piramicin, 0.025% (w/v) ampicillin, 0.001% (w/v) rifampicin] at 26°C, under conditions of 16h light and 8h darkness (Tuite, 1969). After two weeks, in order to collect the inoculum necessary for each experimental unit, the mycelium on the surface of ten Petri dishes was removed with a sterilized scalpel blade, mixed with 100 mL sterilized Milli-Q water and blended two times for 4 sec.

Description of the compartmentalized microcosms and experimental setting

The compartmentalized system used was described in detail in chapter V. The microcosms (subunits A, B and C) used in this experiment were built using polyethylene plates (Plastroph, St-Etienne-de-Lauzon, QC) in order to compartmentalize the soil. A 48 µm nylon membrane (Sefar America, Buffalo, NY) which permits microbial but not root growth between compartments was glued with silicone adhesive sealant (Superflex Clear RTV, Loctite, Mississauga, ON) on one side of subunits C. Subunits A and/or B (used for plant inoculation) were assembled as required by the experimental treatments by placing subunits C in-between for separation. The microcosms were closed with full plates and supported with screws, bolts, eyelets and filing clamps.

In the first step, an acetate was glued on each side of subunits A by the use of silicone sealant in order to isolate every compartment. Depending on the treatment, the

acetate was cut on one side of the compartments to permit tomato plant colonization with AMF (treatments Gi and Gm) or it was left intact to block the growth of AMF towards the plants (treatment G-). The subunits A were then placed between two subunits B containing leek plants either colonized with *G. intraradices* (on one side) or with *G. mosseae* (on the other side), and tightly joined together as described previously. The tomato seedlings were transferred in the subunits A and thinned to one seedling per compartment. Two subunits A were prepared for each experimental unit. Two weeks after planting and until harvest, 2 mL of root exudates (collected from tomato plants colonized with *G. mosseae*, with *G. intraradices* or non-colonized) or sterilized Milli-Q water (Milli-Q synthesis, RiOs^{TM}, Millipore, Mississauga, ON) were applied daily on the soil of each compartment of subunits A. After three weeks from seedlings inoculation, required for mycorrhizal colonization, subunits B containing leek plants were taken away and subunits A were inoculated with *P. nicotianae* as described below.

In order to prepare the central compartment used as a source of *P. nicotianae* inoculum, a 150 µm nylon membrane was glued on both sides of a subunit B, using silicone sealant. Twelve tomato seedlings were sown and grown for one week, before a 100 ml suspension of *P. nicotianae* mycelium was evenly spread on the surface of the substrate. Two weeks later, the infected tomato plants were thinned and the subunit was incorporated in the experimental system. The acetates on each side of subunits A containing tomato plants previously inoculated with AMF or treated with root exudates were replaced by new ones. An opening was cut facing the appropriate compartments to allow the growth of *P. nicotianae* towards the tomato plants (treatment P+) or the acetate was left intact to block the pathogen growth towards the plants (treatment P-). Each subunit A containing the tomato plants was joined to a second subunit A (then

called intermediary subunit) only filled with gravel and growth substrate. The subunit B inoculated with *P. nicotianae* was then set in-between the two such subunits A constructions.

Mycorrhizal inoculation of tomato plants

To ensure a fast and homogenous mycorrhizal colonization, tomato plants were grown in subunits A leaned to subunits B holding leek plants already colonized with either *G. intraradices* or with *G. mosseae*. In each subunit B, leek seedlings were sown and thinned to six plants. After two weeks, each subunit B was opened and a water suspension of 500 viable spores of *G. intraradices* or *G. mosseae* were poured on the sub-apical zone of each plantlet root system. After seven weeks, root colonization was verified (as described below) on a root subsample from each subunit B then ready to be used to inoculate tomato plants.

Production of tomato root exudates

Root exudates of tomato plants colonized with *G. intraradices*, *G. mosseae* or without mycorrhizal colonization were collected, as described in chapter V. Briefly, subunits B containing leek plants colonized with either *G. intraradices*, *G. mosseae* or non-AM inoculated were placed between two subunits A and the whole assemblage was tightly joined together as described previously. One inoculated tomato seedling was allowed to grow into each compartment of subunits A. After five weeks, the root system of each tomato plant was gently washed under tap water and incubated in Erlenmeyer flasks filled with 100 mL sterilized Milli-Q water, for 22h, in the same greenhouse as the main experiment. Solutions were successively passed through Whatman No. 4 and

No. 42 filter papers and then 0.22 μm nitrocellulose filters (Millipore). The root exudates were concentrated to a ratio of 1 g of root fresh weight equivalent to 20 mL of exudate solution by lyophilization. The pH of the solutions (and of sterilized Milli-Q water used as a control) was then adjusted to 6.0 before sterilization by filtration through 0.22 μm nitrocellulose filters and storage at -20°C until use. The extent of mycorrhizal root colonization of the plants was later assessed as described below.

Plant harvest

After six weeks of growth, all the growth substrate was withdrawn from each compartment of subunits A containing tomato plants. The root system was separated from the soil using sterilized forceps and gloves. The whole soil from each experimental unit was then immediately placed in individual sterilized plastic bags (Fisher Scientific, Ottawa, ON) and frozen at -20°C.

DNA extraction and PCR amplification

Each soil sample was homogenized before the total DNA was extracted from a 0.6 g subsample using the UltraClean Soil DNA Isolation Kit (MoBio Laboratories Inc., Solana Beach, CA), according to the manufacturer's instructions. A nested Polymerase Chain Reaction of the 16S rRNA gene was performed using first the universal primers pA and pH (Edwards et al., 1989) and then the primers 341F+GC and 534R, to amplify the V3 region (Muyzer et al., 1993) (Table VI). All PCRs were conducted in 50 μL volumes. The soil genomic DNA (for the first run) or the amplification products (for the second run) were diluted to 1/100 and 2 μL were added to 1 μL of primers (10μM, Alpha DNA, Montreal, QC), 1 μL of dNTPs mix (10mM), 0.5 μL of bovine serum

albumin (BSA, 100X, New England Biolabs, Ipswich, MA), 5 μL of 10 × PCR buffer and 2.5 μL of dimethyl sulfoxid (DMSO, Sigma-Aldrich, Oakville, ON, for the second run only). The PCR amplifications were performed in a TC-514 thermal cycler (Techne Inc., Princeton, NJ) and consisted in an initial denaturation at 95°C for 5 min, after which the temperature was adjusted to 80°C and 1.25 U of Taq DNA Polymerase (TAQ PCR core kit, Qiagen, Mississauga, ON) were added. Then, for the first run, 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 90 sec, and a final extension at 72°C for 5 min were performed. The second run consisted in a Touch down protocol with decreasing annealing temperatures from 65°C to 55°C for 35 cycles (Muyzer et al., 1993).

Table VI. Sequences of the primers used for the nested-PCR amplifications of the bacterial 16S rRNA gene

Primer	Region amplified	Sequence	Reference
pA	Entire 16S	5'-AGAGTTTGATCCTGGCTCAG-3'	Edwards et al., 1989
pH	Entire 16S	5'-AAGGAGGTGATCCAGCCGCA-3'	Edwards et al., 1989
341 F + GC	V3	5'CGCCCGCCGCGCGCGGCGGGCG GGGCGGGGGCACGGGGGGCCTAC GGGAGGCAGCAG-3'	Muyzer et al., 1993
534 R	V3	5'-ATTACCGCGGCTGCTGG-3'	Muyzer et al., 1993

DGGE profiling of the bacterial community

DGGE analysis was performed using the Dcode Universal Mutation Detection System (Bio-Rad, Missauga, ON) according to the manufacturer's instructions except that the Model 485 Gradient Former was used for the gel preparations instead of the Model 475. Twenty µL of PCR product were charged in each well and the electrophoresis was run for 16 h at 60V (at 60°C) on a 8% acrylamide/bis-acrylamide (37.5:1) gel with a 40-70% denaturant gradient, where 100% denaturant corresponded to 7 M of urea and 40% (v/v) formamide. A molecular marker (described below) was loaded in the first and the last well of the DGGE gels to facilitate gel-to-gel comparisons. The gel was stained 15 min in 1× SYBR Gold (Invitrogen, Carlsbad, CA), digitized using a Chemi-Doc apparatus (BioRad) and analyzed using the Quantity One 4.0 software (BioRad). The banding patterns were binary coded to obtain a taxa presence-absence matrix used for statistical analyses.

In order to prepare the molecular marker, two bands from a DGGE gel performed with one experimental block were collected with a sterilized scalpel and deposited in 50 µL TE 10mM buffer, pH 7.6. The DNA was extracted by incubation at 50°C for 10min, crushing with a microcentrifuge tube pestle and centrifugation at 10 000 g for 1 min. The V3 region was then reamplified (as described above). Pure cultures of *Escherichia coli* (XL1-blue Mrf, Stratagene, Cedar Creek, TX) and of bacterial isolates 10D, 10G and 60A (cf. chapter VII) were also used. Each isolate was incubated in Tryptic Soy Broth (TSB, Quélab), in Erlenmeyer flasks. After 72h of incubation in Tryptic Soy Broth (TSB, Quélab, QC), 3 mL of culture broth were transferred and incubated in new Erlenmeyers filled with 27 mL of TSB. When OD_{600} was between 0.65 and 0.9, the

total genomic DNA of 3 mL of culture broth was extracted by two successive treatments in 1.5 mL 1 M NaCl each followed by a centrifugation at 16 000 rpm for 4 min and removal of the supernatant. The extracted DNA was then suspended in TE 10 mM buffer, pH 7.6 and stored at -20°C (Versalovic et al., 1994). The V3 region of the 16S rRNA gene was then directly amplified as described above, except that no BSA and DMSO were incorporated in the PCR mix. Equal volumes of each PCR product were then mixed and charged as molecular markers in DGGE gels.

Statistical analyses

To describe bacterial taxa associations with mycorrhizal inoculation, exudates application and *Phytophthora* inoculation treatments, discriminent analysis was performed on the taxa presence-absence matrix of DGGE banding patterns using the CANDISC procedure of the SAS/STAT software system for Windows (release 8.02, SAS Institute Inc. 2004, Cary, N.C.). Rare and common bands types were excluded because of the distortion they provoke in this type of ordination. This type of diagram allows interpretation of the distance between centroid points for individual samples. The significance of differences between the twelve treatments on bacterial communities were then determined by a Fisher test of the Mahalanobis distances between treatment clusters.

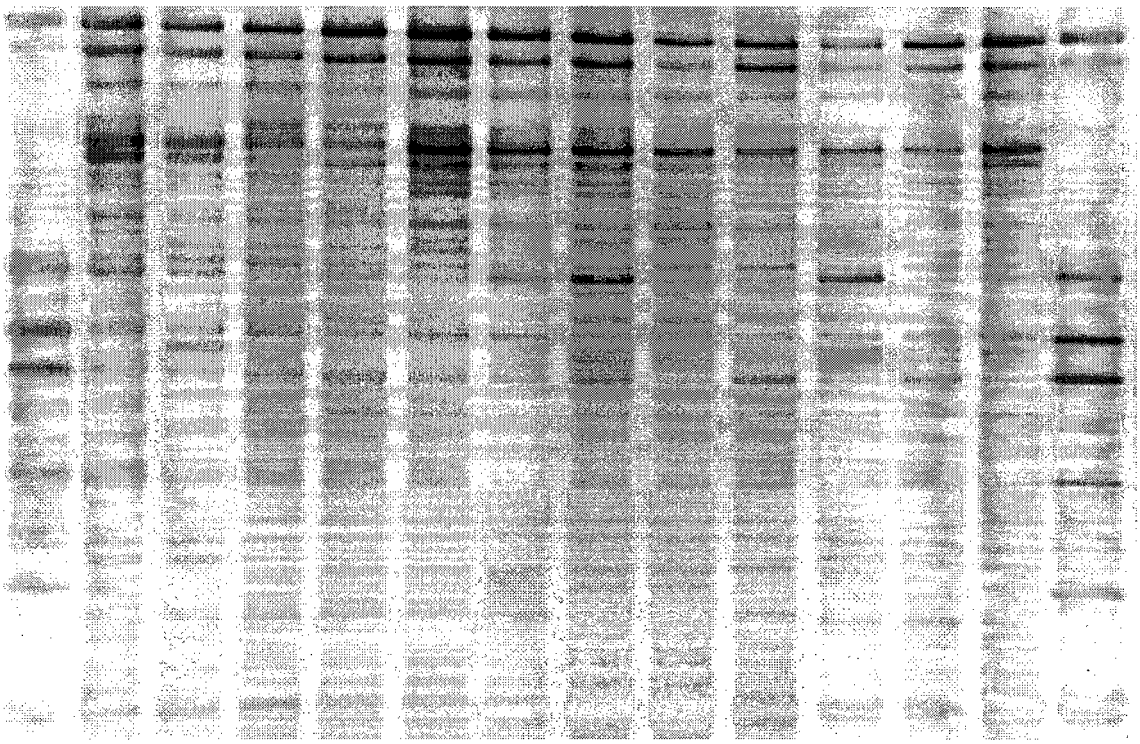
Correspondence analysis were also performed on the taxa presence-absence matrix of DGGE banding patterns using the CORRESP procedure of SAS (release 8.02, SAS Institute Inc.) with the exclusion rare and common bands types as well.

Results

A typical DGGE profile obtained from the rhizosphere of tomato plants after six weeks of growth is shown in Fig.16. A total of 60 different bands were detected, with numbers varying from 26 to 38 per sample. Of these, 25% occurred in all samples, while 42% were treatment-specific. Direct inoculation of the mycorrhizal fungi had the largest influence on the bacterial taxa assemblage within the tomato rhizosphere (Fig. 17 and Table VII). The total number of bands observed on the DGGE gels was higher when *G. mosseae* was inoculated, with 44 bands in the presence and 49 bands in the absence of *P. nicotianae*, in comparison to the other treatments. Moreover, this treatment is the one that led to the apparition of the highest number of highly specific bands when *P. nicotianae* was co inoculated with this AMF. At the opposite the smallest number of bands observed was on control treatments without mycorrhizal inoculation nor exudate application, with only 38 bands in the presence of *P. nicotianae* and 39 in its absence (Table VII).

A canonical correspondence analysis of the effect of the experimental treatments on the sequence variant frequencies revealed that the two first factors that mostly changed the bacterial community within the tomato rhizosphere were root colonization with *G. mosseae* and with *G. intraradices* (data not shown). The two principal components of a discriminent analysis of the bacterial 16S rRNA gene fragments profile (Fig. 17) described 67% of the variation in the data set. This analysis procedure reduced the different band location variables to a smaller set of hypothetical variables, or principal components (PC), that are ranked for their importance in describing variation in the data set. The first principal component (PC1 plotted on the *x* axis in Fig.

Figure 16. Typical DGGE gel of bacterial 16S gene sequence variants detected from tomato rhizosphere, after six weeks of growth.



S 1 2 3 4 5 6 7 8 9 10 11 12 S

Lanes S, Molecular markers composed of (from top to bottom) two unidentified bacteria, *Escherichia coli* (XL1-blue Mrf, Stratagene, Cedar Creek, TX), isolate 60A, *E. coli* (XL1-blue Mrf, Stratagene), isolates 10G, 10D and 10D again; 1. E-G-P-; 2. E-GiP-; 3. E-GmP-; 4. E^{G-}G-P-; 5. E^{Gi}G-P- ; 6. E^{Gm}G-P- ; 7. E-G-P+; 8. E-GiP+ ; 9. E-GmP+ ; 10. E^{G-}G-P+; 11. E^{Gi}G-P+ ; 12. E^{Gm}G-P+.

E-, control with sterilized water and without exudates application.

E^{G-}, application of root exudates collected from non mycorrhizal tomato plants.

E^{Gi}, application of root exudates collected from plants colonized with *G. intraradices.*

E^{Gm}, application of root exudates collected from plants colonized with *G mosseae.*

Gi, plants inoculated with *G. intraradices*, Gm: plants inoculated with *G. mosseae.*

G-, plants non AMF inoculated.

P+, plants inoculated with *P. nicotianae*.

P-, plants non inoculated with the pathogen.

Table VII. Effect of mycorrhizal inoculation, application of exudates from mycorrhizal roots and Phytophthora inoculation on the 16S rRNA gene profiles of rhizosphere bacteria of six week old tomato plants

Exudate application[1]	AMF inoculation[2]	Phytophthora inoculation[3]	Total number of bands[4]	Bands present in all repetitions[5]	Number of specific bands[6]
Control	Non myc	Control	39	43.3 %	1
Control	*G. intra*	Control	42	36.6 %	3
Control	*G. moss*	Control	44	35.0 %	0
Control	Non myc	*P. nicot*	38	36.6 %	0
Control	*G. intra*	*P. nicot*	41	30.0 %	0
Control	*G. moss*	*P. nicot*	49	31.6 %	4
Exu non myc	Non myc	Control	43	41.6 %	0
Exu *G. intra*	Non myc	Control	40	35.0 %	1
Exu *G. moss*	Non myc	Control	40	35.0 %	0
Exu non myc	Non myc	*P. nicot*	42	36.6 %	0
Exu *G. intra*	Non myc	*P. nicot*	42	38.3 %	0
Exu *G. moss*	Non myc	*P. nicot*	42	43.3 %	1
Total			60		

[1] Control: application of Milli-Q water; Exu non myc: exudates collected from non mycorrhizal plants; Exu *G. intra*: exudates collected from plants colonized with *G. intraradices*; Exu *G. moss*: exudates collected from plants colonized with *G. mosseae*.

[2] Non myc: plants non-AMF inoculated; *G. intra*: plants inoculated with *G. intraradices*; *G. moss*: plants inoculated with *G. mosseae*.

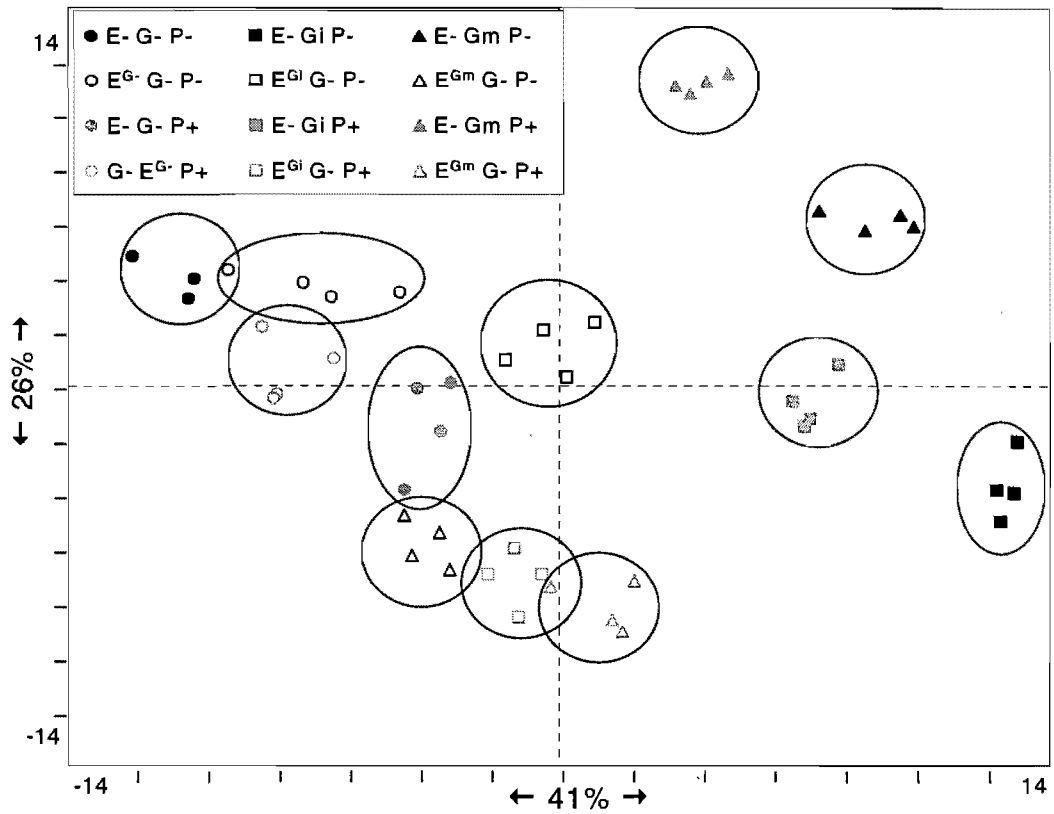
[3] Control: plants non inoculated with *Phytophthora nicotianae*; *P. nicot*: plants inoculated with *P. nicotianae*.

[4] Total number of bands detected at least one time.

[5] Percentage of bands detected in all repetitions of each treatment.

[6] Number of bands specifically present or absent within each treatment.

Figure 17. Discriminant analysis of 16S rRNA gene profiles of bacterial taxa associated with the rhizosphere of six week old tomato plants.



E-, control with sterilized water and without exudates application.

E^{G-}, application of root exudates collected from non mycorrhizal tomato plants.

E^{Gi}, application of root exudates collected from plants colonized with *G. intraradices*.

E^{Gm}, application of root exudates collected from plants colonized with *G mosseae*.

Gi, plants inoculated with *G. intraradices*,

Gm, plants inoculated with *G. mosseae*.

G-, plants non AMF inoculated.

P+, plants inoculated with *P. nicotianae*.

P-, plants non inoculated with the pathogen.

17) described 41% of the variation between the bacterial community profiles. An additional 26% of the variation was described by PC2, plotted on the *y* axis. The ordination diagram plotted with respect to these two vectors revealed a closed similarity between rhizosphere bacterial communities of control plants (without AMF, *P. nicotianae* and exudate enrichment) and of non mycorrhizal plants filled with exudates from non mycorrhizal plants, either when the plants were inoculated or not with *P. nicotianae*. A similar closed similarity was noticed between rhizosphere bacterial communities of plants inoculated with *P. nicotianae* only and plants inoculated with exudates from plants colonized with *G. intraradices* and inoculated with *P. nicotianae*, or with exudates from plants colonized with *G. mosseae* and inoculated or not with *P. nicotianae*. At the opposite, the points representing the rhizosphere bacterial communities of plants directly inoculated with *G. mosseae* or with *G. intraradices*, either inoculated or non inoculated with *P. nicotianae*, formed clusters that were clearly separated from the other treatments and from each other. The statistical differences between the bacterial communities described by PC1 and PC2 were also examined by analysis of Mahalanobis distances between clusters. By this analysis, the rhizosphere bacterial community of the control plants (non inoculated with AMF and *Phytophthora*, and without exudate enrichment) were statistically different from rhizosphere communities of plants inoculated only with *G. intraradices* ($P<0.05$) or with *G. mosseae* ($P<0.1$). Meanwhile, no significant difference was measured between these three treatments when *P. nicotianae* was inoculated. The rhizosphere bacterial communities of plants inoculated with the two mycorrhizal species presented no significant difference between each other, with or without *P. nicotianae* inoculation. No significant difference was neither observed between rhizosphere bacterial communities

of non-AMF plants filled with exudates from non-mycorrhizal plants or from plants colonized either with *G. intraradices* or *G. mosseae*, with *P. nicotianae* inoculation or not. The application of exudates from non-mycorrhizal plants on control plants (non inoculated with AMF and *Phytophthora*, and without exudate enrichment) did not induce a significant modification of the bacterial community. Mahalanobis distances between clusters were not significant between the direct inoculation of *G. intraradices* and the application of exudates from plants colonized with this mycorrhizal species, but was significant between the direct inoculation with *G. mosseae* and the supply of exudates from plants colonized with the same mycorrhizal species ($P<0.1$). These assessments could be done on plants inoculated and non-inoculated with *P. nicotianae*. The application of *P. nicotianae* did not change the community of control plants, nor the community of plants colonized with either *G. intraradices* or with *G. mosseae*, or filled with exudates (from non-AMF inoculated plants or from plants colonized with *G. intraradices* or with *G. mosseae*).

Discussion

The PCR-DGGE analysis of the 16S rRNA gene fragments permitted to confirm that a direct tomato root colonization with either *G. mosseae* or with *G. intraradices* significantly modified the bacterial community structure within the rhizosphere after six weeks of growth. Meanwhile, we showed for the first time that the application on the tomato rhizosphere of exudates from tomato plants colonized with the same AMF did not lead to significant changes of the bacterial community structure. According to the discriminant analysis, the bacterial community was not significantly different between the *G. mosseae* and *G. intraradices* mycorrhizosphere. Tomato rhizosphere was analysed after six weeks of growth. At that time the influence of the two studied AM species may not be yet significantly different. Using the same PCR-DGGE approach, Marschner and colleagues (2001) previously brought to light differences among the rhizosphere bacterial community between non-mycorrhizal maize plants colonized with *G. mosseae* or with *G. intraradices* and non-colonized plants. These differences were root-zone specific and were more important after seven than after four weeks of growth. The two AM fungi species had similar bacterial communities after four weeks but these differed after seven weeks. These authors also observed similar trends with *G. intraradices* and *G. deserticola* inoculated on canola (considered as a non-mycorrhizal species), on clover and on two different genotypes of tomato (Marschner and Timonen, 2005). After plant genotype, mycorrhizal inoculation was the reported factor to mostly influence soil-microbial community, and a plant-genotype-fungus interaction was also described suggesting that mycorrhizal impact depends on the plant species studied. Wamberg et al. (2003) also observed the apparition and disappearance of bands in a

DGGE analysis performed on the rhizosphere of pea plants after *G. intraradices* inoculation.

The high microbial activity present in the rhizosphere in comparison to the bulk-soil was often believed to be due to the supply of nutrients liberated by the roots in the form of exudates. Soil or rhizosphere enrichment with artificial exudates shifted the microbial community structure more and more consistently as substrate concentration loaded increased (Baudoin et al., 2003; Griffiths et al., 1999; Kozdrój and van Elsas, 2000; Pennanen et al., 2004). It is possible that, in this study, the quantity of exudates supplied on tomato rhizopshere was not high enough to induce differences between exudates from mycorrhizal and non-mycorrhizal plants on the bacterial community. Moreover exudates were collected after five weeks only after sowing. It is possible that they were not enough different, at that harvest time, to significantly affect the rhizosphere bacterial community. Nonetheless, using a similar exudate concentration as we used here, Pinior et al. (1999) showed that root exudates from cucumber plants colonized with *G. mosseae* inhibited further root colonization with this AMF, in comparison to water-treated controls. This effect was later shown to be systemic as exudates (still used at the same concentration) collected from non-mycorrhizal roots of mycorrhizal plants induced similar results (Vierheilig et al., 2003). The absence of effects we noticed here would therefore probably not be related to inadequate exudate concentration. Moreover, Lynch and Whipps (1990) calculated that exudates of barley and maize contained only 9% and 10% of the amount of substrate required to explain the quantified microbial biomass in their respective rhizosphere. Lugtenberg and colleagues (1999) reported that the ability of the biocontrol bacteria *Pseudomonas fluorescens* WCS365 to use sugars does not play a major role in tomato root

colonization. They showed that the mutant PCL1083 from WCS365 impaired in the ability to grow on simple sugars reached the same population levels at the root tip as the wild-type strain, when inoculated on germinated tomato seeds. Additionally, it was demonstrated that the bioavailability of some tested amino acids detected in tomato exudates is too low to support root tip colonization by auxotrophic mutants of *P. fluorescens* strain WCS365. The genes required for amino acid synthesis are therefore necessary for root colonization (Simons et al., 1997). Therefore, the establishment of the rhizosphere (and of the mycorrhizosphere) may not only be simply related to the supply of exudates.

On the other hand, it has been shown that roots constitute an essential physical support for the rhizosphere competence of specific soil bacteria. Mutants of *Pseudomonas chlororaphis* strain PCL1391 impaired in the known tomato root colonization traits (motility and production of the site-specific recombinase) were not able to control *F. oxysporum* f. sp. *radicis-lycopersici* at the opposite to the wild-type strain (Chin-A-Woeng et al., 2000). Root colonization thus plays a crucial role in biocontrol for bacteria showing that the presence of AMF structures would also be essential for bacterial competence within the mycorrhizosphere and for their possible contribution to biocontrol. Physical interactions between bacteria and AMF have been previously described. Bacterial adherence to spores and/or hyphae of several AMF species, under sterile conditions, was reported to depend on the bacterial strain and on the fungal species and vitality (Artursson and Jansson, 2003; Bianciotto et al., 1996a; Levy et al., 2003; Toljander et al.,2006). The first stages of attachment (non-receptor dependant) would be governed by general physiochemical parameters, such as electrostatic attraction and then later secured by specific bacterial cell surface

components. The capacity to adhere to *G. intraradices* structures by different bacterial species was shown to depend on the capacity to form biofilms as mutants affected in the production of extracellular polysaccharides (EPS) essential for biofilm formation were strongly impaired in the capacity to attach to both AM root and AMF structures (Bianciotto et al., 2001a). Furthermore, mucoid mutants of the biocontrol strain *Pseudomonas fluorescens* CHAO (with an alginate biosynthesis activation) adhered more importantly to the surface of this fungus than the wild type strain (Bianciotto et al., 2001b). Bacterial saprophytic activity was suggested by scanning electron microscopy observations of *G. geosporum* spores (Roesti et al., 2005). The spore's outer layer was shown to be eroded and to be covered by mucilaginous products suggesting that AMF are directly consumed by bacteria. Thus, AMF may specifically favour the proliferation of some bacteria serving as substrate or interacting with them inducing the formation of biofilms.

G. mosseae was shown to increase the population of the biocontrol agent *P. fluorescens* CHAO within the tomato and leek rhizosphere (Edwards et al., 1998). Nonetheless, *G. intraradices* had a negative effect on the population of *P. fluorescens* DF57 both in the rhizosphere and the mycosphere in association with cucumber (Ravnskov et al., 1999). The bacteria were not attached to the AMF hyphae or to spores either on *in situ* filters (after inoculation of the bacteria in a root-free compartment of mycorrhizal plants) or after attachment assays performed *in vitro* by dual-incubation. The authors postulated that competition for inorganic nutrients other than P could explain their results. The overall decrease of microbial activity after roots colonization by AMF has also been previously suggested to be due to competition for substrates (Christensen and Jakobsen, 1993; Raiesi and Ghollarata, 2006).

We previously identified bacteria belonging to the three genera *Paenibacillus*, *Bacillus* and *Methylobacterium* within surface disinfected spores of the *G. mosseae* isolate used in the present study (cf. chapter VII). *Paenibacillus* B2 was also previously isolated from *G. mosseae* mycorrhizosphere (Budi et al.,1999). In the present study, the *G. mosseae* inoculum was obviously accompanied with associated bacteria that have probably proliferated as the AMF was developing, while this was not the case for *G. intraradices* as this species was inoculated from axenic *in vitro*-produced spores. The bacteria accompanying *G. mosseae* would have been then detected among those identified by the PCR-DGGE we performed. They also could have interacted with other microorganisms modifying that way the soil bacterial community structure.

In our study, contrarily to the two AMF species, the inoculation of *P. nicotianae* did not significantly modify the rhizosphere bacterial community structure. Moreover, the impact of AMF on the bacterial community was not affected by the inoculation of *P. nicotianae*. AMF were inoculated three weeks before *P. nicotianae*. Their biomass and then their influence on the rhizosphere bacterial community may for this reason be more important. Moreover, *P. nicotianae* strain we used did not induce significant root necrosis three weeks after inoculation (cf. chapter V). The capacity of necrotrophy of this pathogen may be related to its influence on the rhizosphere bacterial community. Contrarily, AMF, in symbiosis with roots, may have a more important impact on this data. Similarly, the bacterial community analyzed by PCR-DGGE in hydroponic systems used for tomato growth was not importantly perturbed by the introduction of *Phytophthora cryptogea* or of *Pythium aphanidermatum* (Calvo-Bado et al., 2006). The authors proposed that the microbial communities that established early and became dominant in the soilless growing systems they used was robust and resistant to

perturbation such as pathogen introduction. The growth substrate we used was inoculated with a soil filtrate AMF-free two weeks before planting. This microflora was also probably stable and poorly affected by pathogen inoculation. Using the same analytical method, Yang et al. (2001) showed that the rhizosphere bacterial community of avocado plants infected with the pathogen *P. cinnamomi* and receiving repetitive applications of the biocontrol agent *P. fluorescens* st. 513 was similar to the rhizosphere of healthy plants but significantly different from the rhizosphere of plants infected with the pathogen only. Pathogens such as *P. nicotianae* may have a reduced impact on the rhizosphere microflora in comparison to biocontrol agents. If AMF stimulate some biocontrol agents within the mycorrhizosphere, the establishment of these antagonists which would then control efficiently the pathogen proliferation would be poorly affected by pathogen infection.

The application of exudates from plants colonized with *G. mosseae* or with *G. intraradices* on the tomato rhizosphere did not reduce the proliferation of *P. nicotianae* whereas the direct root colonization with these AMF did (cf. chapter V). The exudates from AMF-colonized plants did not neither significantly modify the rhizosphere bacterial community in the present study while direct mycorrhizal colonization did. Therefore, our results suggest that if the biocontrol mediated by AMF is related to the formation of a bacterial community then unfavourable to pathogen proliferation within the rhizosphere, these modifications would not occur through changes of root exudate composition after mycorrhizal colonization. Biocontrol may not be determined by the modification of root exudation after mycorrhizal colonization. Especially, AMF structures may harbour specific bacteria or serve as a niche propitious to the formation of a microbial community antagonistic to such pathogens. Some isolates of especially

Bacillus simplex, *B. niacini* and *B. drententis,* isolated from spores of *G. mosseae*, showed various levels of antagonism against *P. nicotianae*, *F. solani* and different isolates of *F. oxysporum, in vitro* (cf. chapter VII). Budi et al. (1999) also showed *Paenibacillus* B2, isolated from this AMF mycorrhizopshere, could induce antagonism over *P. nicotianae, in vitro*. This isolate also reduced tomato root necrosis caused by this pathogen, displayed cellulolytic, proteolytic, chitinolytic and pectinolytic activities and was shown to exude the antibiotic polymixin B1 and two other polymyxin-like compounds (Budi et al., 1999; 2001; Selim et al., 2005). Nonetheless, the increase of enzyme synthesis and cell wall modifications involved in local but also systemic defense mechanisms following tomato colonization with *G. mosseae* have been described (Cordier et al., 1996; 1998; Pozo et al., 1996; 1998; 1999; 2002a). Therefore, biocontrol mediated by AMF would be also importantly related to the stimulation of the plant defense mechanisms.

Conclusion

The results presented here suggest that AMF may impact the rhizopshere microbial diversity structure mainly through other mechanisms than the direct liberation of exudates or the modification of root exudation of the host plant. The AM fungi may limit the proliferation of some microorganisms through direct competition for space and nutrients. They may also serve of nutritional resource or of habitat. Moreover, the impact of mycorrhizal colonization on the microbial diversity was, in this study, poorly affected by inoculation with *P. nicotianae* suggesting that if biocontrol agents are stimulated by AMF, this effect would be stable in time and weakly affected by pathogen infection. It is likely that biocontrol mediated by AMF is the consequence of various mechanisms with their relative impact varying with the combination of plant-pathogen-AM species and environmental conditions. Biocontrol would not only be orchestrated by the fungus itself but dependant on the relationships it maintains with other soil microorganisms. Further work is necessary to understand how the mycorrhizosphere is established in order to better describe the mechanisms responsible for biocontrol.

Acknowledgements

We are grateful to Franck Panabières (INRA Antibes) for providing the *P. nicotianae* isolate 201 and to Stéphane Daigle (Institut de recherche en biologie végétale) for performing the discriminent and the correspondance analyses. This work was supported by an NSERC grant allocated to M. St-Arnaud and a FQRNT team grant to M. Jolicoeur.

Chapitre VII

*Bacterial diversity associated with spores of the arbuscular mycorrhizal fungus Glomus mosseae and antagonistic potential against the soilborne pathogens Phytophthora nicotianae, Fusarium solani and F. oxysporum**

Laëtitia Lioussanne, Mario Jolicoeur & Marc St-Arnaud

Les bandes présentes au niveau du gel de DGGE obtenu par l'amplification PCR du gène ribosomal 16S de l'ADN génomique extrait directement à partir des spores désinfectées en surface du *G. mosseae* sont actuellement en cours d'extraction, de clonage et de séquençage dans le but d'identifier les bactéries associées aux spores et d'effectuer des comparaisons avec les isolats identifiés sur milieu de culture standard. Anne Keough sera coauteure de cet article.

Les résultats de ce chapitre ont été présentés lors du congrès indiqué ci-bas et seront prochainement soumis afin de publication dans une revue internationale.

Lioussanne, L., A. Keough, M. Jolicoeur et M St-Arnaud. 2006. Diversity of *Glomus mosseae* spore-associated bacteria and their antagonism over soil-borne pathogens, *in vitro*. *Fifth International Conference on Mycorrhiza*, Granada, Spain, pp. 203.

Abstract

Bacteria associated with the arbuscular mycorrhizal fungi (AMF) may play a significant role on the mycorrhiza-mediated growth promotion of the host plant, as well as in the inhibition of soilborne pathogens. In order to gain insight into the bacterial taxa living in close association with the AMF *Glomus mosseae*, spores were surface-disinfected in 2% chloramine T for various times and the associated bacteria were then identified using two different approaches. Culturable bacteria were isolated after incubation of spores on tryptic soy agar and identified using the 16S rRNA gene. Genomic DNA was extracted directly from the spores, the 16S rRNA gene was amplified, amplification products were separated by DGGE and each sequence variant was identified by sequencing. Eighteen bacterial isolates were obtained and classified into nine phylogenetically different groups belonging to three genera: *Methylobacterium*, *Bacillus* and *Paenibacillus*. Thirty-four sequence variants were found by PCR-DGGE suggesting that bacterial diversity associated with the spores is wider than the isolated taxa. The antagonism of the culturable bacterial isolates among the soilborne pathogens *P. nicotianae* isolate 201, *F. solani* MT-240 and *F. oxysporum* MT-104, MT-118 and MT-119 was tested *in vitro*. Fourteen isolates showed various levels of antagonism, especially isolates identified as *B. simplex* but also as *B. niacini*, *B. drententis*, *Paenibacillus* sp. and *Methylobacterium* sp. This antagonism was stronger against *P. nicotianae* than against the other pathogens.

Keywords

Glomus mosseae, antagonism, soilborne pathogens, arbuscular mycorrhiza-associated bacteria, DGGE.

Introduction

Arbuscular mycorrhizal fungi (AMF) have been shown to reduce disease development in a wide number of plant-pathogen associations (St-Arnaud and Vujanovic, 2007). Root colonisation with the AMF *Glomus mosseae* has been shown to alleviate the pathogenic effects of different soilborne pathogens: *Verticillium dahliae* (Karagiannidis et al., 2002), *Fusarium solani* (Dar et al., 1997), *Rhizoctonia solani* (Abdalla and Abdel-Fattah, 2000; Berta et al., 2005) and *Aphanomyces euteiches* (Slezack et al., 1999; 2000). The control of *Phytophthora nicotianae* was particularly studied, in tomato plants (Cordier et al., 1996; Pozo et al., 2002a; Trotta et al., 1996; Vigo et al., 2000). Trotta and colleagues (1996) postulated that the better assimilation of P in mycorrhizal plants would not be involved in the reduction of root necrosis whereas the stimulation of plant defense mechanisms that coincides with *G. mosseae* root colonization was described (Cordier et al., 1996; 1998; Pozo et al., 2002a). Nonetheless, Vigo et al. (2000) observed that the number of infection loci formed by infection with *P. nicotianae* was reduced in tomato roots colonized by this AMF in comparison to non-mycorrhizal roots which suggests that the development of the pathogen would be limited before it penetrates the roots. We showed (cf. chapter V) that root exudates collected from *G. mosseae*-colonized tomato roots and applied to uncolonized roots challenged with *P. nicotianae* did not inhibit the intraradical growth of the pathogen similarly to direct colonization with the AMF, suggesting that the biocontrol would not be determined by a modification in root exudation. It was further shown that the application of mycorrhizal root exudates in the non-mycorrhizal tomato rhizosphere did

not neither modify the structure of the dominant bacterial community, whereas direct inoculation with *G. mosseae* did (cf. chapter VI).

Various bacteria (especially *Paenibacillus* and *Bacillus*) have been isolated from the mycorrhizosphere of plants colonized with *G. mosseae* (Andrade et al., 1997; Citernesi et al., 1996) or with other AMF (Secilia and Bagyaraj, 1987; Andrade et al., 1997; Mansfield-Giese et al.; 2002, Artursson and Jansson, 2003). A high number of bacteria and bacterium-like organelles were also directly observed not only within non disinfected sporocarps of *G. mosseae* (Filippi et al., 1998; MacDonald et al., 1982) but also from spores or mycelium of other AMF (MacDonald et al., 1982; Mayo et al., 1986; Roesti et al., 2005; Xavier and Germida, 2003). Moreover, on the basis of morphological data and 16S rRNA gene sequences, *Candidatus* Glomeribacter gigasporarum was proposed as a new taxon of *Burkholderiaceae* for a non culturable (thus obligatory) bacterial endosymbiot of spores (within the vacuoles), mycelium and clover intraradical hyphae of *Gigaspora margarita* (Bianciotto and Bonfante, 2002; Bianciotto et al., 1996b; 2000; 2003). This endobacteria was later phenotypically described in details (Jargeat et al., 2004) and shown to be widespread within *Gigasporaceae* (Bonfante, 2003). This taxa appears to be vertically transmitted (Bianciotto et al., 2004) and to contain nitrogen fixation genes instead of *G. margarita*, suggesting that this AMF might fix nitrogen and then deliver it to the symbiotic plant through the associated bacterial population (Minerdi et al., 2001).

It has been estimated that from 0.1% to 10% of the microorganisms found on typical agricultural soils are culturable using current culture media formulations (Head et al., 1998; Hill et al., 2000; Theron and Cloete, 2000; Torsvik et al., 1990). At the opposite, culture independent methods based on 16S rRNA gene permit the extraction

and the analysis of over 90% of the microorganisms that can be observed microscopically *in situ* (Hill et al., 2000). Denaturent gradient gel electrophoresis (DGGE) is a method by which fragments of DNA of the same length but of different sequence can be resolved electrophoretically. PCR products on gradient gels can be later sequenced and the resultant information used to infer about the microbial diversity of the original sample (Head et al., 1998; Muyzer, 1999; Muyzer and Smalla, 1998; Theron and Cloete, 2000). The combination of both culture-independent using PCR products of the 16S rRNA gene and culture-dependent techniques has been shown to provide very useful and complementary information about the structure of microbial communities (Dunbar et al., 1999; Garbeva et al., 2001; Hengstmann et al., 1999; Kaiser et al., 2001; Smit et al., 2001).

In order to test the implication of bacteria associated with *G. mosseae* in the biocontrol mediated by this AMF, we first identified the bacteria present within surface disinfected spores of *G. mosseae* by isolation on a culture medium and by PCR-DGGE of total genomic DNA extracted from the spores. We later tested *in vitro* the antagonism of the culturable bacteria over different soilborne pathogens (*P. nicotianae* isolate 201, *F. solani* MT-240 and *F. oxysporum* MT-104, MT-118 and MT-119).

Materials and Methods

Biological material and growth conditions

Glomus mosseae (T.H. Nicolson & Gerd.) Gerd. & Trappe (BEG 12) was routinely grown in pots containing leek plants (*Allium porrum* L, cv. Farinto; Société coopérative agricole du sud de Montréal, Sherrington, QC). The growth substrate was composed of 1/3 sandy loam soil withdrawn in a non chemically treated field located in the Montreal Botanical Garden, 1/3 sand (Entreprises Guy Bélanger, St-Henri-Mascouche, QC) and 1/3 perlite, autoclaved twice at 121°C and stored at least for two weeks before use. The leek seeds were surface-sterilized by immersion in 70% (v/v) ethanol for 15 min followed by 25% (v/v) commercial bleach (6% sodium hypochlorite) plus 1% (v/v) Triton X100 for 20 min and three rinses with sterilized distilled water. They were pregerminated for four days, in darkness, on humid filter papers. Seedlings were transferred in pots containing the growth substrate mixed with leek root fragments colonized with *G. mosseae* from a previous culture. The plants were maintained in a growth chamber under conditions of 16 h light (22°C) and 8 h darkness (20°C). The soil was supplied with Long Ashton nutrient solution as needed (Hewitt, 1966).

Phytophthora nicotianae Breda de Haan (isolate 201) was routinely grown on autoclaved V8 agar [V8 juice (Campbell Company, Toronto, ON), diluted 1:10 in Milli-Q water, 0.2% (w/v) $CaCO_3$, 1% (w/v) gellan gum (Gel Gro, ICN Biochemical,Cleveland, Ohio), 0.005% (w/v) piramicin, 0.025% (w/v) ampicillin, 0.001% (w/v) rifampicin], at 26°C, under conditions of 16h light and 8h darkness (Tuite, 1969). *Fusarium solani* (Mart.) Sacc. (MT-240) and *F. oxysporum* Schltdl. (MT-

104, MT-118 and MT-119: IRBV, Université de Montréal) were grown on Potato Dextrose Agar (Difco, Becton, Dickinson and Company, MD).

Surface disinfection of *G. mosseae* spores and isolation of bacteria

Spores of *G. mosseae* were recovered from four-months old leek pot cultures with more than 50% of root length bearing mycorrhizal colonization, as estimated after staining with ink and vinegar (Vierheilig et al., 1998). The spores were isolated by blending the growth substrate and root fragments in tap water, wet sieving through 500- and 45-μm meshes and decanting. The presence of spores in the suspension was verified at 25× magnification, under a dissecting binocular. Further spore purification was carried out by two successive centrifugations at 3000 rpm, for 2 min in a density gradient with a 60% (w/v) sucrose layer at the bottom. The spores were collected from the gradient interface with a syringe and thoroughly washed with sterilized distilled water. They were then slowly vortexed in sterilized distilled water plus one drop of Tween 80 and washed abundantly in sterilized distilled water, three times successively, before storing overnight at 4°C. Remaining debris and sand were removed with a micropipette and sterilized forceps. The spores were then separated into four subsamples each submitted to a specific disinfection time in chloramine T: 10 min, 20 min, 40 min and 60 min. A Buchner filtration system fit out with a Whatman No. 4 filter paper was used for spore surface disinfection. Incubations and rinses were carried out while gently shaking the spore suspension with a sterilized spoon. The spores were first immersed in 2% (v/v) chloramine T (in sterilized distilled water), with one drop of Tween 80, for 5, 10, 20 or 30 min (for treatments with 10 min, 20 min, 40 min and 60

min of incubation respectively), rinsed three times in 2% (v/v) chloramine T and incubated again in this solution until the final treatment time was reached. They were then rinsed in sterilized distilled water first quickly and abundantly and then 10 times, for one min each. One hundred spores per treatment time were finally spread, using a micropipette, on Petri dishes (with ten spores per Petri dish) filled with Tryptic Soy Agar (TSA, Difco, Bacton, Dickinson and Company, MD) and incubated upside down in the dark, at 26°C. Another hundred spore subsample per treatment was collected for an immediate total genomic DNA extraction.

Identification of bacterial culturable isolates

Each bacterial colony growing on the TSA dishes in association with *G. mosseae* spores was streak on new Petri dishes (previously filled with TSA) until they visually contained only one isolate, based on their appearance in culture. Each isolate was incubated in Tryptic Soy Broth (TSB, Quélab), in Erlenmeyer flasks. After 72 h, 3 mL of culture broth were transferred and incubated in new Erlenmeyer flasks filled with 27 mL of TSB. When OD_{600} was between 0.65 and 0.9, the total genomic DNA of 3 mL of culture broth was extracted by two successive treatments in 1.5 mL NaCl (1 M) each followed by a centrifugation at 16 000 rpm, for 4 min, and removal of the supernatant. The extracted DNA was then suspended in TE buffer (10 mM, pH 7.6) and stored at -20°C (Versalovic et al., 1994).

The entire 16S rRNA gene was amplified by PCR (performed in 50 µL volumes) using the pA and pH primers (10 µM, Alpha DNA, Montreal, QC) (Table VI). The DNA extract was first diluted to 1:10 and 2 µL were added to 1 µL of each primer, 1 µL of dNTPs (10mM) and 5 µL of buffer 10X. The PCR amplification was performed in a

TC-514 thermal cycler (Techne Inc., Princeton, NJ) and consisted in an initial denaturation at 95°C for 5 min, after which the temperature was adjusted to 80°C and 1.25 U of Taq DNA Polymerase (TAQ PCR core kit, Qiagen, Mississauga, ON) were added. Thirty cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 90 sec were then applied followed by a final extension at 72°C for 5 min. The PCR product was then commercially sequenced (Genome Quebec, Montreal, QC) using both primers pA and pH to obtain a quasi-total 16S rRNA gene sequence. Sequences were aligned and percentages of similarity between pair sequences were calculated using BioEdit (version 7.0.0). The sequences were afterwards submitted to similarity searches in the GenBank database (http://www.ncbi.nlm.nih.gov/) using the BLASTn search algorithm for identification. A distance tree was then produced with the PAUP* program (version 4.0b10)(Swofford, 2002) using a neighbor joining approach, by incorporating the 16S rRNA gene sequences of the isolated bacteria and of the following organisms for comparison (National Center for Biotechnology Information, Bethesda, MD; Accession numbers and lengths of the sequences are indicated in brackets): *Agrobacterium tumefaciens* (DQ458962, 1451 bp), *Rhizobium leguminosarum* bv. *phaseoli* strain RRE6 (AY946012, 1457 bp), *Streptococcus pneumoniae* (AF003930, 1515 bp), *Azospirillum brasilense* (NCIMB 11860, Z29617, 1461 bp), *Staphylococcus pasteuri* strain AE4-2 (AB269765, 1451 bp), *Pseudomonas syringae* isolate ICMP 3023 (ATCC19310, AJ308316, 1372 bp), uncultured *Aquificales* bacterium clone IBSC-15 (AB197166, 1074 bp), *Candidatus* Glomeribacter gigasporarum (X89727, 1457 bp), *Methylobacterium* sp. (DQ507203, 1140 bp), *Bacillus* sp. (AY635873, 1503 bp; AJ316308, 1503 bp; DQ275185, 1472 bp; DQ314538, 1511 bp and AJ316313, 1502

bp), *Bacillus firmus* (DQ514315, 1513 bp), *Bacillus simplex* (DQ275175, 1498 bp and AJ628745, 1503 bp), *Bacillus drententis* (DQ275176, 1494 bp), *Bacillus niacini* (AB021194, 1526 bp), *Paenibacillus* sp. (AY745242, 1443 bp; AM162319, 1500 bp), *Paenibacillus favisporus* (AY308758, 1547 bp), *Paenibacillus cineris* (AJ575658, 1505 bp), *Paenibacillus rhizosphaerae* (AY751754, 1547 bp), *Paenibacillus ginsengagri* (AB245383, 1469 bp) and *Paenibacillus lautus* (AB073188, 1505 bp). Statistical support in the tree was assessed via bootstrap analysis (1,000 replicates). The phylogenetic tree was edited using Treeview (Win32).

Identification of bacteria by PCR-DGGE

For DNA extraction, the *G. mosseae* spores were first suspended in 80 µL of 10 mM Tris-HCl (pH 7.5), heated at 90°C for 10 min (in a TC-514 thermal cycler), crushed with a plastic pestle, treated with proteinase K (final concentration 100 µg/mL) at 65°C for 15 min and then at 50°C for 3 h, and heated again at 90°C for 10 min. The DNA was afterwards precipitated in 200 µL ethanol 100% (v/v) and 10 µL sodium acetate (3 M, pH 5.2) for 20 min at -20°C. After 30 min of centrifugation at 14 000 rpm, the supernatant was removed and 1 mL of ethanol 70% (v/v) was added before a second centrifugation at 14 000 rpm, for 5 min. The ethanol was then taken over and once dried, the DNA was suspended in 20 µL of TE (10 mM, pH 7.6)(Bianciotto et al., 1996b).

A nested PCR targetting the partial bacterial 16S rRNA gene was performed using the universal primers pA and pH in the first run, and then the primers 341F+GC and 534R (to amplify the region V3, Table VI). All PCRs were conducted using the same conditions described above, except that DNA or amplified products were not diluted

and 0.5 µL of bovine serum albumin (BSA, 100X, New England Biolabs, Ipswich, MA) and 2.5 µL of dimethyl sulfoxide dimethyl sulfoxide (DMSO, Sigma-Aldrich, Oakville, ON, in the second PCR run only) were also added. The first PCR run was performed as followed: a denaturation at 94°C for 30 s, annealing for 30 s (with annealing temperatures of 60°C for the first five cycles, 55°C for the next five cycles, and 50°C for the last 25 cycles) and elongation at 72°C for 4 min (Bianciotto et al., 1996b). The second run consisted in a Touch down protocol with decreasing annealing temperatures from 65°C to 55°C for 35 cycles (Muyzer et al., 1993). The DGGE was performed as described in chapter VI using a DCode Universal Mutation Detection System (BioRad, Missauga, ON), according to the manufacturer's instructions except that the Model 485 Gradient Delivery System instead of the Model 475 was used for the gel preparations. Briefly, 20 µL of PCR product was charged in each well and the electrophoresis was run for 16 h at 60 V (at 60°C) on a 8% acrylamide/bis-acrylamide (37.5:1) gel with a 40-70% denaturant gradient, where 100% denaturant corresponded to 7 M of urea and 40% (v/v) formamide. The gel was stained with SYBR Gold 1× (Invitrogen, Carlsbad, CA), digitized using a Chemi-Doc apparatus (BioRad) and analyzed using the Quantity One 4.0 software (BioRad).

In vitro fungal inhibition assays

Three mL aliquots of liquid cultures of each bacterial isolate were incubated in Erlenmeyer flasks containing 27 mL of TSB. When OD_{600} was between 0.65 and 0.9, a sterilized filter paper disc (Millipore Corporation, Bedford, MA, AP15, Ø 7 mm) was soaked in the liquid broth and placed at 5 mm from the periphery of a Petri dish filled

with 25 mL of V8 agar. Two discs soaked in a bacterial isolate and one control disk immersed in sterilized TSB were inoculated on each Petri dish, equidistant from each other. After 72 h of incubation, a 1 cm diameter gel disk of 10 day-old *P. nicotianae* culture was inoculated in the center of the Petri dish. The inhibition of *P. nicotianae* growth was estimated after four days and classified into four cases, according to the diameter of inhibition zone (DI, in mm): 0, no inhibition; 1, slight inhibition (0<DI<2); 2, intermediary inhibition (2<=DI<5); 3 high inhibition (DI>=5). The same method was used with *F. solani* and the three *F. oxysporum* isolates except that the biotests were performed on TSA and the bacteria were inoculated at the same moment as the fungi. The tests were performed five times per combination of bacterial and fungal isolates.

Results

Identification of culturable bacterial isolates

Eighteen bacterial isolates grew from *G. mosseae* spores on TSA: 10 after 10 min, 3 after 20 min and 5 after 60 min of disinfection in 2% chloramine T, while no bacterial growth was observed after 40 min of treatment. The isolates were named according to the treatment time and then in alphabetic order. The 16S rRNA gene sequences from GenBank and presenting the highest percentages of similarity with the isolates all belong to the three following eubacteria genera: *Methylobacterium* (Proteobacteria; Alphaproteobacteria; Rhizobiales; Methylobacteriaceae), *Paenibacillus* (Firmicutes; Bacilli; Bacillales; Paenibacillaceae) and *Bacillus* (Firmicutes; Bacilli; Bacillales; Bacillaceae). According to the pairwise percentage of similarity between the 16S rRNA gene sequences, the highest percentage of similarity with sequences registered in GenBank (Table IIX) and the phylogenetic consensus distance tree (Fig. 18), the isolates were pooled into nine phylogenetically distinct groups (or clusters). The 16S rRNA gene of all isolates was successfully PCR amplified and fragments from 1443 bp (10H) to 1348 bp (10J) were obtained and sequenced. Nonetheless, isolate 10I presented a gap between the bases 745 and 825. Moreover, isolates 10D and 10I had >10% of undefined bases (N) and presented less than 96.0% and 98,8% similarity with *Paenibacillus* spp., respectively. They were thus classified into the phylogenetic groups 4 and 7. Because of the unsatisfactory sequencing, they were not included in the distance tree and were not considered in the following analysis.

The 16S rRNA gene sequences all presented percentages of similarity over 99% with sequences registered in GenBank, except for 60E presenting 98.5% identity with

Table IIX. Sequence analysis of 16S rRNA gene of bacterial isolates from surface sterilized spores of *Glomus mosseae*

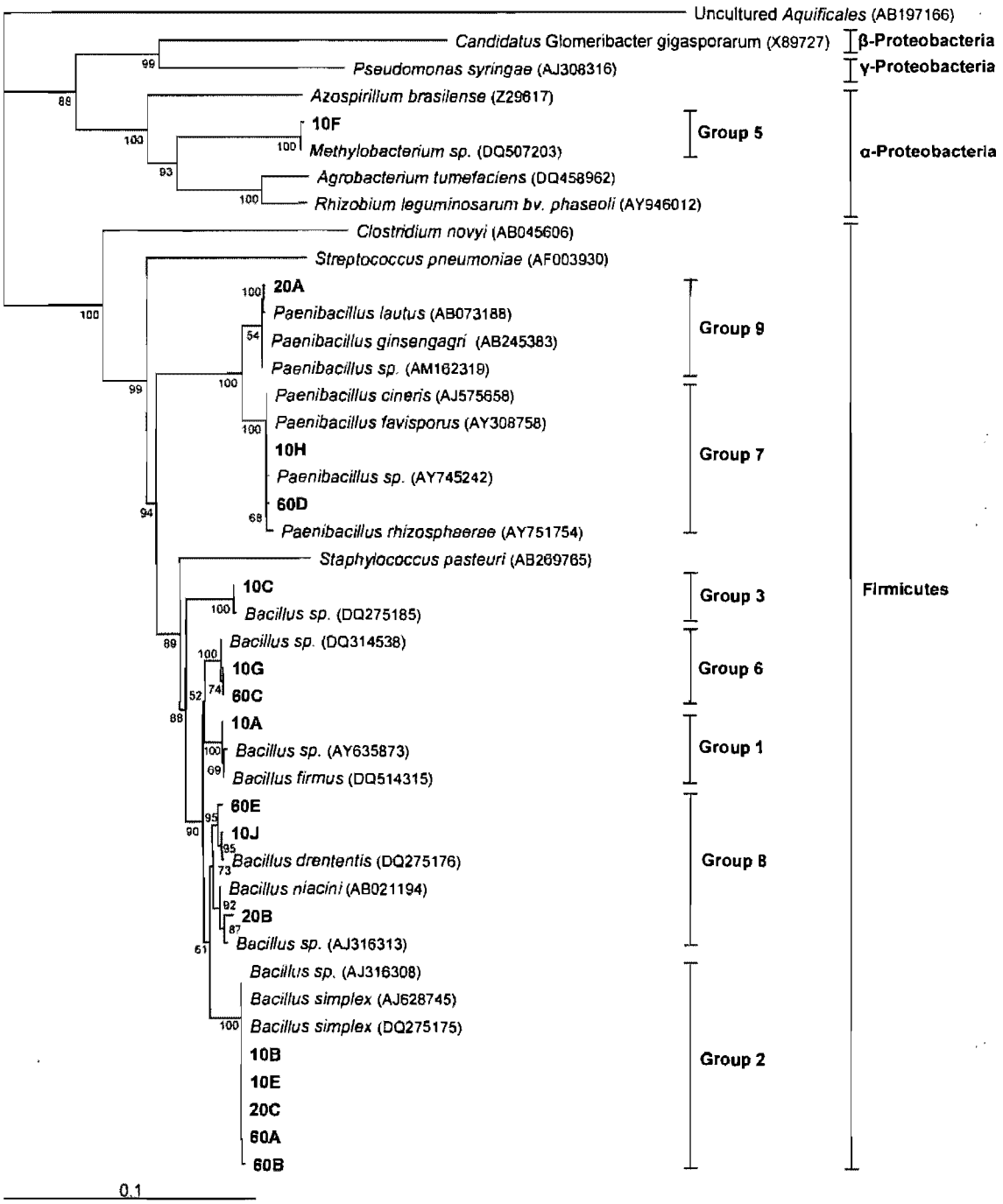
Isolate[1]	Group[2]	Most closely related taxa	Similarity[3]	Accession number[3]
10A	1	*Bacillus* sp.	99.9%	AY635873
		Bacillus firmus	99.4%	DQ514315
10B	2	*Bacillus* sp.	99.9%	AJ316308
		Bacillus simplex	99.9%	DQ275175 - AJ628745
10C	3	*Bacillus* sp.	99.8%	DQ275185
10D	4	*Paenibacillus* sp	96.0%	AB043866
10E	2	*Bacillus* sp.	99.9%	AJ316308
		Bacillus simplex	99.9%	DQ275175 - AJ628745
10F	5	*Methylobacterium* sp.	99.9%	DQ507203
10G	6	*Bacillus* sp.	99.6%	DQ314538
10H	7	*Paenibacillus favisporus*	99.7%	AY308758
		Paenibacillus cineris	99.6%	AJ575658
		Paenibacillus sp.	99.6%	AY745242
		Paenibacillus rhizosphaerae	99.6%	AY751754
10I	7	*Paenibacillus* sp.	98.8%	AY745242
		Paenibacillus favisporus	98.8%	AY308758
		Paenibacillus cineris	98.6%	AJ575658
10J	8	*Bacillus drententis*	99.2%	DQ275176
		Bacillus niacini	98.3%	AB021194
		Bacillus sp.	98.2%	AJ316313
20A	9	*Paenibacillus ginsengagri*	99,8%	AB245383
		Paenibacillus sp.	99.7%	AM162319
		Paenibacillus lautus	99.2%	AB073188
20B	8	*Bacillus* sp.	99.1%	AJ316313
		Bacillus niacini	98.9%	AB021194
		Bacillus drententis	98.5%	DQ275176
20C	2	*Bacillus* sp.	99.9%	AJ316308
		Bacillus simplex	99.9%	DQ275175 - AJ628745
60A	2	*Bacillus* sp.	99.9%	AJ316308
		Bacillus simplex	99.9%	DQ275175 - AJ628745
60B	2	*Bacillus* sp.	99.9%	AJ316308
		Bacillus simplex	99.9%	DQ275175 - AJ628745
60C	6	*Bacillus* sp.	99.6%	DQ314538
60D	7	*Paenibacillus favisporus*	99.7%	AY308758
		Paenibacillus cineris	99.6%	AJ575658
		Paenibacillus sp.	99.6%	AY745242
		Paenibacillus rhizosphaerae	99.5%	AY751754
60E	8	*Bacillus drententis*	98.5%	DQ275176
		Bacillus niacini	97.8%	AB021194
		Bacillus sp.	97.6%	AJ316313

[1] The isolates were named according to the duration of surface-disinfection treatment in 2% (v/v) chloramine T (in minutes) and then in alphabetic order.

[2] The isolates were pooled in distinct phylogenetic groups according to the percentage of similarity between each other and with strains registered in GenBank.

[3] Percent similarity and accession number of sequences with closest match in GenBank.

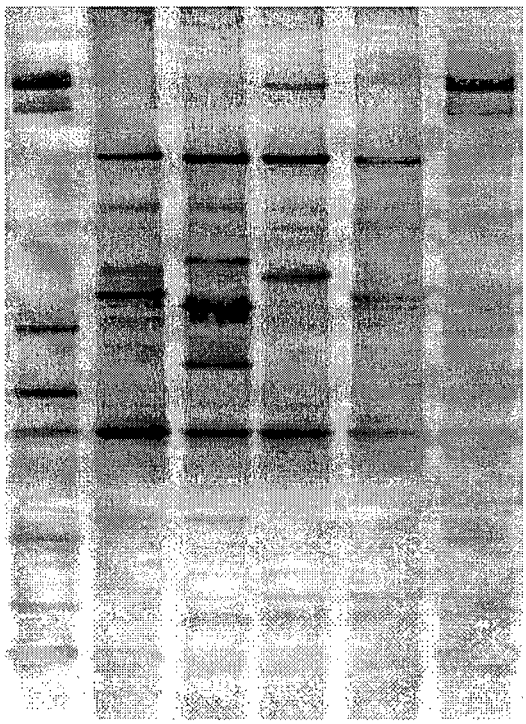
Figure 18. Phylogenetic consensus tree based on the alignment from the 16S rRNA gene sequence of culturable bacterial isolates obtained from surface-disinfected *Glomus mosseae* spores.



Reference 16S rRNA gene sequences from GenBank were incorporated in the tree for comparison. Statistical support of the tree was assessed via bootstrap analysis (1,000 replicates). An uncultured *Aquificales* bacterium was used as an outgroup. The phylogenetic classification of the isolates in different groups is indicated.

B. drententis (DQ275176) and 97.8% with *B. niacini* (AB021194). Isolate 10A (99.4% identity with *Bacillus firmus* DQ514315) presented only 96.9% similarity with 10G (the most closely related isolate, according to the distance tree) and was then placed alone into group 1. Isolates 10H and 60E had 99.9% similarity, 10B and 60A, 99.7%, 10E and 60A 99.9%, 20C and 60A 100%, and 60B and 60A 99.8% between each other. These isolates were placed into group 2 and all presented a high similarity with *B. simplex* (DQ275175 - AJ628745). Isolate 60A possessed only 95.9% and 95.7% similarity with 10J and 20B respectively (classified into group 8). Isolate 10C was distant from all other isolates with a percentage of similarity that never exceeded 94.5%. The highest similarity this isolate presented with sequences from the GenBank database was 98% with *Bacillus* sp. (DQ275185). It was consequently placed alone into group 3. Isolate 10F, classified alone into group 5, presented 99.9% identity with *Methylobacterium* sp. (DQ507203). Isolates 10G and 60C had 99.9% similarity between each other and a high similarity with *Bacillus* sp. (DQ314538); they were classified into group 6. Isolate 10G presented only 96.7% and 96.3% similarity with 10J and 20B, respectively. Isolates 20B and 60D presented 97.5% similarity between each other and a high similarity with *P. favisporus* (AY308758), *P. cineris* (AJ575658), *Paenibacillus* sp. (AY745242) and *P. rhizosphaerae* (AY751754). They were classified into group 7. Isolates 20B and 10J had 98.4% similarity between each other, 60E and 10J 98.5% and 20B and 60E 98.7% so they were classed into group 8 with a high similarity (>99%) with *B. drententis* (DQ275176), *B. niacini* (AB021194) and *Bacillus* sp. (AJ316313). Isolate 20A, classified alone into group 9, presented 99.8% similarity with *P. ginsengagri* (AB245383), 99.7% *with Paenibacillus* sp. (AM162319) and 99.2% with *P. lautus* (AB073188), while it presented only 95.0% similarity with 10H from group 7.

Figure 19. Typical DGGE gel of bacterial 16S gene sequence variants detected following direct DNA extraction from surface-disinfected spores of *Glomus mosseae*.



Lanes S, molecular markers composed of (from top to bottom) two unidentified bacteria, *Escherichia coli* (XL1-blue Mrf, Stratagene, Cedar Creek, TX), isolate 60A, *E. coli* (XL1-blue Mrf, Stratagene), isolates 10G, 10D and 10D again.

Lane 10, surface-disinfection of *G. mosseae* spores for 10 min.

Lane 20, surface-disinfection of *G. mosseae* spores for 20 min.

Lane 40, surface-disinfection of *G. mosseae* spores for 40 min.

Lane 60, surface-disinfection of *G. mosseae* spores for 60 min.

Identification of bacteria by PCR-DGGE

The DGGE profile obtained from surface disinfected spores of *G. mosseae* is shown in Fig. 19. A total of 34 different bands were detected, with 6 being represented in all treatments. Sixteen bands were observed after 10 and 20 min of surface disinfection, while 17 and 13 were noticed after 40 and 60 min of surface disinfection respectively. Two bands were specific to treatments of 10 and 20 min, 6 bands were specific to 40 min and 4 bands were specific to 60 min of treatment with chloramine T.

***In vitro* soilborne pathogen inhibition**

Among all bacterial isolates recovered from surface disinfected spores of *G. mosseae*, 14 showed various levels of antagonism *in vitro* against the tested soilborne pathogens. The intensity of antagonism varied with the bacterial isolate and the pathogen species/isolate tested (Table IX). The bacterial isolates from group 2 (*B. simplex*) all induced a high level of antagonism over all the tested soilborne pathogens. This antagonism was higher against *P. nicotianae* (with the highest score of 3.0 from isolate 10B) and smaller against *F. oxysporum* MT-119 (with a maximum score of 1.4 from 10B and 20C). Isolate 10C (*Bacillus* sp., group 3) showed the same pattern of antagonism with scores of 3.0 against *P. nicotianae* and 1.2 against *F. oxysporum* MT-119. Isolates from group 7 (*P. favisporus*, *P. cineris* and *P. rhizosphaerae*) were moderately antagonistic against the tested pathogens. The antagonism level never exceeded 1.8 but was always higher against *P. nicotianae*. Isolates from group 8 (*B.*

Table IX. Antagonism of the different bacterial isolates recovered from spores of *Glomus mosseae* against *Phytophthora nicotianae*, *Fusarium solani* and *F. oxysporum* (isolates MT-104, MT-118 and MT-119) *in vitro*

Bacterial isolate	Group[1]	Growth inhibition index[2]				
		P. nicotianae	*F. solani*	*F.o.* 104	*F.o.* 118	*F.o.* 119
10A	1	0.0	0.0	0.0	0.0	0.0
10B	2	3.0	1.4	1.8	1.4	1.4
10E		2.0	1.8	1.8	1.8	1.0
20C		1.6	0.8	1.2	2.2	1.4
60A		2.8	1.8	1.8	1.4	0.8
60B		2.2	1.2	1.6	2.0	0.8
10C	3	3.0	1.4	1.8	1.4	1.2
10D	4	0.0	0.0	0.0	0.0	0.0
10F	5	1.0	0.0	0.0	0.0	0.0
10G	6	0.0	0.0	0.0	0.0	0.0
60C		0.0	0.0	0.0	0.0	0.0
10H	7	1.1	0.0	0.0	0.0	0.0
10I		1.2	1.4	1.4	1.4	0.0
60D		1.8	0.6	1.2	1.2	1.2
10J	8	1.2	0.2	0.4	0.4	0.2
20B		0.0	0.4	0.4	0.4	0.0
60E		0.0	0.0	0.0	0.8	0.0
20A	9	0.4	0.0	0.4	0.0	0.4
Control		0.0	0.0	0.0	0.0	0.0

[1] Phylogenetic grouping of the isolates as defined in Table IIX.

[2] Growth inhibition score is the mean of five replicates. Growth inhibition was estimated after four days of growth on V8 agar for *P. nicotianae* and on TSA for *F. solani* and the three *F. oxysporum* isolates. Inhibition was estimated into four cases, according to the diameter of inhibition (DI, in mm): 0, no inhibition; 1, slight inhibition (0<DI<2); 2, intermediary inhibition (2<=DI<5); 3 high inhibition (DI>=5).

niacini and *B. drententis*) and group 9 (*P. ginsengagri*, *P. lautus*) exerted a reduced antagonism that never exceeded 1.2 for isolate 10J against *P. nicotianae* and 0.4 for isolates 20A and 20B. Isolate 10F (group 5, *Methylobacterium* sp.) induced showed antagonism only over *P. nicotianae* with a score of 1.0. Isolates 10A (group 1, *B. firmus*), 10D (group 4) and all isolates from group 6 (*Bacillus* sp.), as well as the negative control, did not show any antagonism over the tested pathogens.

Discussion

The eighteen isolates recovered from surface-disinfected spores of *G. mosseae* on a standard bacterial culture medium were shown by sequencing of the 16S rRNA gene to belong to three genera: *Bacillus*, *Paenibacillus* and *Methylobacterium*. *Methylobacterium* sp. 10F was isolated only once, from the 10 min disinfection treatment, while *Bacillus* spp. and *Paenibacillus* spp. isolates were abundantly recovered from 10 min, 20 min and 60 min chloramine T incubation treatments. The isolates from group 2 (*B. simplex*) in particular, but also isolates identified as *B. niacini*, *B. drententis*, *Paenibacillus* spp. and *Bacillus* sp. exerted various levels of antagonism over the tested soilborne pathogens. This antagonism was always stronger against *P. nicotianae* while its intensity depended on the isolate of *F. oxysporum* tested. By PCR-DGGE from the 16S rRNA gene from the total genomic DNA directly extracted from the spores, 34 bands were observed suggesting a higher diversity and the presence of bacterial taxa not recovered by culturable method.

Taxonomists of bacteria define a species as strains holding a minimum of 70% DNA similarity by reassociation and a minimum of 97% sequence identity of the 16S rRNA gene (Stackebrandt and Goebel, 1994; Stackebrandt et al., 2002; Wayne et al., 1987). Here, isolate 10A (group 1) was clearly identified as *B. firmus*, and isolates 10B, 10E, 20C, 60A and 60B (group 2) as *B. simplex* (Heyrman et al., 2005). On the other hand, isolate 10C (group 3), 10G and 60C (group 6) were only identified to the genus level *Bacillus*, and isolate 10F to the genus *Methylobacterium*. The identification of new isolates based only on 16S rRNA gene sequencing presents obvious limits. For example, Rivas et al. (2005) reported that 16S rRNA gene sequences of isolates from *P.*

rhizosphaerae showed 99.6% and 99.4% similarity with *P. cineris* or *P. favisporus* respectively, but that DNA-DNA hybridization values were 45% and 42%, confirming that these isolates were closely related but distinct to these species. 16S rRNA gene similarity between isolates 10J, 60E, 20B, *B. drententis* and *B. niacini* was over 99%. However, 16S rRNA gene sequencing alone did not allow to determine if these isolates belong to the species *B. drententis* or *B. niacini* which were shown to be closely related (Felske et al., 2004). Isolates 10J, 60E and 20B were therefore classified into the common group 8. Nonetheless, isolates 10J and 60E presented a higher homology with *B. drententis* while isolate 20B showed a higher homology with *B. niacini*. Similarly, we can not determine if isolates 10H and 60D (group 7) belong to the species *P. favisporus*, *P. cineris* or *P. rhizosphaerae* nor if isolate 20A belongs to the species *P. ginsengagri* or *P. lautus*. In this work, isolates that were grouped in the same cluster showed a similar antagonism score against the different soilborne pathogens tested, supporting the classification we performed.

Paenibacillus, *Bacillus* and *Methylobacterium* taxa were previously identified in plant mycorrhizosphere or from soil AMF structures. About 80% to 92% of the bacteria isolated from decontaminated spores of *G. clarum* NT4 were *Bacillus* (Xavier and Germida, 2003). Nonetheless, *Methylobacterium radiotolerans* was recovered by these authors from untreated spores of this mycorrhizal species. By bromodeoxydurine (BrdU) immunocapture in soil known to contain AMF and RFLP analysis, Artursson and Jansson (2003) also mostly identified *Bacillus* and *Paenibacillus* taxa. *Paenibacillus* was largely identified by FAME analysis in sterilized root free sand extracts after *G. intraradices* inoculation (representing a mycosphere) but not in the AMF mycelium free control (Mansfeld-Giese et al., 2002).

The bacteria we identified in association with *G. mosseae* spores would participate to the limitation of the expend of not only *P. nicotianae* but also of other soilborne pathogens like *F. oxysporum* and *F. solani* within the soil and then within the roots contributing in this manner to the biocontrol mediated by *G. mosseae*. Soil bacteria have been shown to impact pathogen proliferation through direct liberation of toxic compounds, competition for space and nutrients, reduction of Fe and Mn availability, modification of the plant hormone balance and stimulation of plant defense mechanisms (Azcón-Aguilar and Barea, 1996; Bowen and Rovira, 1999; Nehl et al., 1997; Vassilev et al., 2006). Isolates from group 2 (*B. simplex*) induced a high antagonism against the three tested soilborne pathogenic species. An important antagonism from 10C (*Bacillus* sp.) was also noticed. Isolates from group 7 (*B. niacini* or *B. drententis*) showed a moderate antagonism (with a higher antagonism from isolate 10J). Methanol-extracted toxic substances from *B. simplex* and *B. firmus* were previously shown to be mitochondriotoxic, inhibiting the mobility of boar spermatozoa, and to induce vacuolation in Hep-2 cells (Taylor et al., 2005). Isolates of *B. firmus* are commercialized as bio-nematicides to control root-knot nematodes (*Meloidogyne* spp.)(Giannakou et al., 2004). Here, isolate 10A identified as *B. firmus* did not show any antagonism over the tested soilborne pathogens, nor the other *Bacillus* spp isolates 10G and 60C from group 6. Isolates that belong to the genus *Paenibacillus* (groups 7 and 9) showed a moderate antagonism against the tested pathogens. *Paenibacillus* antagonism over different soilborne pathogens (Beatty and Jensen, 2002; Dijksterhuis et al., 1999; Piuri et al., 1998; von der Weid et al., 2005) and insects (Pettersson et al., 1999) has been described, as well as its production of antibiotics and bacteriocins (Beatty and Jensen, 2002; Chung et al., 2000; Mavingui and Heulin, 1994; Piuri et al.,

1998; Rosado and Seldin, 1993; Walker et al., 1998). *P. illinoisensis* induced the biological control of *Phytophthora capcisi*. It activated PR proteins (β-1,3-glucanase, cellulase and chitinase) in leaves but provoked their reduction within roots of pepper plants infected by this pathogen (Jung et al., 2005). Hong and Meng (2003) also purified a β-1,3-glucanase from *Paenibacillus* sp. with antifungal activity against *Pythium aphanidermatum* and *Rhizoctonia solani*. The reported increased activity of β-1,3-glucanase in tomato roots colonized with *G. mosseae* and related with higher resistance to *P. nicotianae* infection (Pozo et al., 1999; 2002a) might therefore be the result of *Paenibacillus* bacteria associated with this AMF. Polymixin B1 and two other polymyxin-like compounds were purified from *Paenibacillus* sp. strain B2 isolated from the mycorrhizosphere of *G. mosseae* (Selim et al., 2005). This strain was previously shown to induce antagonism over *P. nicotianae in vitro*, reduced tomato root necrosis caused by this pathogen, displayed cellulolytic, proteolytic, chitinolytic and pectinolytic activities, increased the root and shoot fresh weights of mycorrhizal tomato plants and stimulated tomato root colonization by *G. mosseae* (Budi et al., 1999; 2000). By phylogenetic comparison of the 16S rRNA gene sequence and analytical profile index (API) system, *Paenibacillus* sp. B2 was shown to constitute a new *Paenibacillus* species that possessed a high similarity with *P. polymyxa*. Here, isolate 10F identified as *Methylobacterium* sp. exerted a reduced antagonism over *P. nicotianae* only. As far as we know, biocontrol mediated by *Methylobacterium* taxa over plant pathogens has never been reported. Nevertheless, antagonism was, here, studied *in vitro*, in culture media favouring specifically each tested pathogen development. This antagonism may be stronger in soil, as nutrients should be more limitant to these pathogens in these conditions. As *Paenibacillus* sp. B2 did not only show antagonism among *P. nicotianae*

in vitro but also on tomato plants growing in the greenhouse (Budi et al., 1999; 2000), the isolates of our study might be antagonist *in vivo* too.

The bacteria we isolated may also be involved in the plant growth promotion frequently observed after AMF colonization with *G. mosseae* or in the stimulation of the AM fungus itself as Mycorrhiza Helper Bacteria (MHB). Germination of surface-disinfected spores of *G. versiforme* was higher in the presence of bacteria previously isolated from non-surface disinfected spores or in the absence of antibiotics application suggesting that associated bacteria stimulate mycorrhizal growth (Mayo et al., 1986). Furthermore, Rillig et al. (2005) provided an evidence that microorganisms associated with inactivated AMF increased soil aggregation known to favour plant growth. *Paenibacillus* members have been shown to act as Plant Growth Promoting Rhizobacteria (PGPR) (Lebuhn et al., 1997; Nielsen and Sørensen, 1997; Ryu et al., 2005) and to increase soil aggregation (Bezzate et al., 2000). Two strains of *P. validus* were shown to act as MHB as they stimulated the hyphal growth of *G. intraradices* up to the formation of full size fertile spores (Hildebrandt et al., 2006). Moreover, *B. firmus* YA5 and *B. pabuli* LA3 isolated from surface disinfected spores of *G. clarum* NT4 stimulated their hyphal growth (Xavier and Germida, 2003). Nonetheless, shoot dry weight and shoot N content of mycorrhizal pea plants was enhanced by inoculation with *B. pabuli* LA3 only. *Methylobacterium* taxa has been shown to stimulate seed germination and plant development, possibly by the production of phytohormones such as cytokinin, indole acetic acid and to form root-nodulating nitrogen-fixing symbiosis with a legume (Hanson and Hanson, 1996; Lidström and Chistoserdova, 2002; Trotsenko et al., 2001).

Conclusion

Isolates recovered from surface disinfected spores of *G. mosseae* and identified in this study as *B. simplex*, *B. niacini*, *B. drententis*, *Paenibacillus* spp. and *Bacillus* sp. were, *in vitro*, antagonistic among different soilborne pathogens (especially among *P. nicotianae* but also among *F. solani* and *F. oxysporum*). Their real implication in the biocontrol mediated *G. mosseae* should be later studied *in vivo*. Further work will be also necessary to investigate the mechanisms implicated in these antagonisms and to answer the question as whether these microorganisms are actually closely associated with the *G. mosseae* spores or are merely dominant soil inhabitants.

Acknowledgements

P. nicotianae (isolate 201) was kindly provided by Franck Panabières, INRA, Antibes, France). We are particularly grateful to Vincent Manzanilla (Insitut de recherche en biologie végétale, Montréal) for his help in the production of the distance tree. This work was supported by an NSERC grant allocated to M. St-Arnaud and a FQRNT team grant to M. Jolicoeur.

Chapitre IIX

Discussion générale

IIX.1. Introduction

La capacité de biocontrôle sur les agents pathogènes du sol des champignons mycorhiziens à arbuscules (CMA) est un phénomène décrit depuis plus de 30 ans (Gerdemann, 1968; 1975) mais dont les mécanismes demeurent encore très mal compris. Afin de tenter de préciser par quels processus les CMA réduisent la prolifération intraracinaire des agents pathogènes et ainsi les symptômes de la maladie provoqués sur la plante hôte, nous avons utilisé le *Phytophthora nicotianae* Breda de Haan (isolat 201) qui présente un mode de prolifération par voie asexuée, induisant la libération abondante de zoospores dont la chémotaxie par les exsudats racinaires peut être étudiée. Le biocontrôle chez la tomate infectée par cet agent pathogène et colonisée par le *Glomus mosseae* (Nicol. et Gerd.) Gerdemann et Trappe (BEG 12) a été démontré à maintes reprises, sans être totalement élucidé.

IIX.2. Le *G. mosseae* mais aussi le *G. intraradices* induisent le biocontrôle du *P. nicotianae*, chez la tomate

Une réduction du nombre de nécroses, une baisse du développement de l'organisme pathogène, ainsi qu'une diminution de l'inhibition de la croissance de l'hôte ont été observés chez la tomate colonisée par le *G. mosseae* (BEG 12) et inoculée avec le *P. nicotianae* (isolat 201) (Cordier et al., 1996; Pozo et al., 2002a; Trotta et al., 1996; Vigo et al., 2000). Dans un système compartimenté, en sol, des plants de tomate ont été colonisés soit par le *G. mosseae* (BEG 12), soit par le *G. intraradices* Schenck et

Smith (DAOM 181 602) par compagnonnage avec des plants de poireau précolonisés par l'un ou l'autre de ces CMA. Les plants de tomate ont ensuite été placés à une distance équivalente d'un système central contenant des racines de tomate inoculées avec le *P. nicotianae* (isolat 201)(cf. chapitre V). La biomasse de cet agent pathogène était significativement réduite dans les racines précolonisées par les CMA par rapport aux racines non-inoculées nous permettant de confirmer l'induction de biocontrôle par le *G. mosseae* et de mettre en évidence, pour le première fois, la capacité de biocontrôle du *G. intraradices* (DAOM 181 602), chez des plants de tomate inoculés par le *P. nicotianae*.

IIX.3. Induction des mécanismes de défense de la plante hôte

La stimulation des mécanismes de défense de plants de tomate colonisés par le *G. mosseae* a été montrée. La synthèse de β-1,3-glucanase synthétisée de façon constitutive est augmentée et des isoformes de chitinase et de β-1,3-glucanase spécifiques ont été détectées dans des racines colonisées par le *G. mosseae* (Pozo et al., 1996; 1998; 1999). Ces changements n'ont pas été détectés de façon systémique même si la réduction du développement de l'agent pathogène et des symptômes de la maladie ont été détectés au niveau de zones racinaires non-colonisées de plants colonisés (Cordier et al., 1996; 1998; Pozo et al., 2002a; Vigo et al., 2000). Cependant, Cordier et al. (1998) ont identifié dans des racines infectées par le *P. nicotianae* non seulement des modifications pariétales locales telles l'accumulation de callose autour des cellules

corticales contenant des arbuscules mais aussi la mise en place de résistance systémique induite (RSI) caractérisée par l'accumulation de protéines de type PR-1 et d'épaississements pariétaux riches en pectines au niveau de racines non-colonisées de plants colonisés par le *G. mosseae*. La stimulation systémique et locale des réactions de défense de la plante hôte semble être un mécanisme important permettant aux CMA d'induire de la mise en place de biocontrôle (St-Arnaud et Vujanovic, 2007). Cependant, une nouvelle isoforme d'activité superoxide dismutase a été détectée suite à la colonisation par le *G. mosseae* mais aussi par le *G. intraradices* (BEG 72) qui n'induit pas la mise en place de bioprotection (Pozo et al., 2002a). De plus, la réduction du nombre de points d'infection observée de façon globale sur les racines de plants de tomate inoculées par le *P. nicotianae* (Vigo et al., 2000) suggère que la stimulation des réactions de défense de la plante hôte n'est pas le seul processus permettant aux CMA de provoquer l'apparition de bioprotection.

L'acide jasmonique (AJ) est une hormone essentielle à la résistance systémique induite (RSI) mise en place par les rhizobactéries (Pozo et al., 2004) qui est accumulée dans les racines mycorhiziennes (Hause et al., 2002; 2007; Isayenkov et al., 2005; Stumpe et al., 2005), de façon non systémique au niveau des racines (Meixner et al., 2005). L'application de l'AJ sur les feuilles de *Tropaeolum majus* et de *Carica papaya* a fortement réduit la colonisation par le *G. mosseae* (Ludwig-Müller et al., 2002). La surexpression du gène *MtAOC1* codant pour l'enzyme AOC (acide jasmonique oxyde cyclase) impliquée dans la synthèse de l'AJ a induit une augmentation du taux d'AJ, mais a réduit le taux de colonisation mycorhizienne (Hause et al., 2007). L'AJ est une molécule impliquée dans les mécanismes de défense suite aux stress biotiques et abiotiques qui s'accumulerait dans les cellules corticales contenant des arbuscules

(Hause et al., 2002) et permettrait la régulation de la symbiose mycorhizienne. Son accumulation provoquée par la symbiose pourrait également être impliquée dans la réduction de la prolifération des agents pathogènes, surtout au niveau des cellules contenant des arbuscules dans lesquelles la présence du *P. nicotianae* n'est jamais observée (Cordier et al., 1996). Aucune étude qui montrerait que les CMA induisent le biocontrôle vis-à-vis des agents pathogènes du sol *via* une chaîne de réactions faisant appel à une accumulation de JA n'a cependant, pour le moment, été publiée.

IIX.4. Les exsudats de racines transformées matures repoussent les zoospores du *P. nicotianae*, *in vitro*

La mise en place d'un système de culture *in vitro* utilisant des boites de Magentas compartimentés nous a permis de collecter des exsudats racinaires de façon simple en filtrant le milieu liquide du compartiment ne contenant pas de glucides lors de l'inoculation (cf. chapitre IV). Des capillaires contenant les exsudats de racines de tomate transformées par l'*Agrobacterium rhizogenes* et cultivées *in vitro* (dans des conditions dépourvues de microorganismes autres que le *G. intraradices*, DAOM 181 602) ont été placés dans des solutions de zoospores du *P. nicotianae* (ATCC 13 196)(également collectées *in vitro*). Lorsque les racines provenaient de cultures matures (âgées de 24 semaines) et étaient colonisées par le *G. intraradices*, les zoospores étaient repoussées (significativement moins attirées que par l'eau et par les exsudats de racines non-colonisées). Le contraire était observé après 16 semaines de culture, les exsudats de

racines colonisées par le CMA étant plus attractifs que les exsudats de racines non-colonisées. La colonisation mycorhizienne entraînerait une modification de la composition des exsudats qui pourrait diminuer le nombre de zoospores entrant en contact avec les racines, un élément clé qui dicte la capacité de prolifération de l'agent pathogène dans une plante hôte. Des modifications de la composition des exsudats racinaires suite à la colonisation par des CMA ont déjà été mesurées suite à une colonisation par différents CMA (Azaizeh et al., 1995; Bansal et Mukerji 1994; Graham et al., 1981; Kapoor et al., 2000; Marschner et al., 1997; Sood, 2003). Les seules modifications que nous avons décelées en comparant la composition en sucres, acides aminés et acides organiques des exsudats que nous avons collectés, *in vitro*, sont une plus grande accumulation de proline dans des exsudats libérés par des racines colonisées par le *G. intraradices* et âgées de 24 semaines par rapport à des racines du non colonisées et/âgées de 16 semaines et des accumulations significativement différentes d'acide isocitrique entre les exsudats de racines mycorhiziennes et non-mycorhiziennes, âgées de 16 et 24 semaines (cf. chapitre IV).

La proline est un acide aminé accumulé dans les tissus végétaux suite à des stress hydriques ou salins et permettant leur protection contre les dommages alors causés (Grote et Claussen, 2001; Kishor et al., 2005; Kuznetsov et Shevyakova, 1999; Rai, 2002; Sharma et Dietz, 2006). Des feuilles de tomate ont accumulé de la proline suite à une infection par le *P. nicotianae* (Grote et Claussen, 2001) ainsi que le cortex de *Threbroma cacao*, en réponse à une infection par le *P. megakarya* (Omokolo et al., 2002). Une accumulation de proline suite à une colonisation par des CMA a été rapportée (Azcón et al., 1996; Diouf et al., 2005; Ruiz-Lozano et al., 1995; Vazquez et al., 2001; Wu et Xia, 2006). La proline pourrait être impliquée dans le biocontrôle

provoqué par les CMA sur les plants de tomate infectés par le *P. nicotianae*. En la libérant, les racines colonisées par les CMA pourraient prévenir l'accumulation de zoospores de l'agent pathogène à proximité des racines dont l'infection serait alors limitée.

La glomaline est une protéine synthétisée par les CMA qui favorise la formation des agrégats du sol (Wright et Upadhyaya, 1998; Wright et al., 1999; Wright et al., 1996). Son impact sur les agents pathogènes n'a jamais été étudié. Les CMA en symbiose avec des racines inhibent la colonisation de ces racines par d'autres CMA, ce de façon systémique (au niveau de racines non-colonisées de plants colonisés) (Vierheilig, 2004; Vierheilig et al., 2000b). La modification des exsudats suite à la colonisation mycorhizienne permettrait la mise en place de cette régulation (Pinior et al., 1999; Vierheilig, 2004; Vierheilig et Piché, 2002; Vierheilig et al., 2003). Il a été montré que des plants mycorhiziens accumulaient des flavonoïdes (de l'acacetine et de la rhamnetine) (Scervino et al., 2005b) ainsi que des isoprenoïdes dérivés des caroténoïdes (la bluménine, la mycorradicine et la nicoblumine)(Fester et al., 1999; 2002a; 2002b; 2005; Strack et Fester, 2006; Vierheilig et al., 2000a). L'application de bluménine sur des racines divisées en système de culture 'split-root' a conduit à la suppression systémique de la colonisation mycorhizienne ultérieure (Strack et Fester, 2006). De plus, des composés dérivés des caroténoïdes isolés d'exsudats de maïs ont été identifiés comme étant les composants responsables de l'inhibition du développement du *Fusarium oxysporum* f. sp. *melongenae* observée chez ces plants de maïs (Park et al., 2004). Ainsi les molécules que nous venons de citer participeraient à la régulation de la symbiose mycorhizienne ainsi que de la mycorhizopshère et pourraient contribuer à

l'inhibition locale et systémique des agents pathogènes induites par la symbiose mycorhizienne (Cordier et al., 1998; Pozo et al., 2002a; Vigo et al., 2000).

IIX.5. Les exsudats de racines colonisées par le *G. mosseae* ou le *G. intraradices* n'induisent par le biocontrôle du *P. nicotianae*, chez la tomate

L'ajout d'exsudats de racines colonisées à des racines de plants de tomate cultivés en sol soit par le *G. mosseae* (BEG 12), soit par le *G. intraradices* (DAOM 181 602) n'a pas induit une diminution de la biomasse intraracinaire du *P. nicotianae* (isolat 201) similaire à celle provoquée par une colonisation directe par ces CMA (cf. chapitre V). L'inhibition de l'attraction des zoospores par les exsudats de racines mycorhiziennes observées *in vitro* ne permettrait pas la mise en place de biocontrôle, lorsque des plants non-transformés sont cultivés en sol. D'autres auteurs ont également observé des effets sur la formation et la germination des propagules de certains agents pathogènes par les exsudats de racines mycorhiziennes. Les exsudats de fraisiers non mycorhiziens ont davantage stimulé la germination du *P. fragariae*, *in vitro*, que les exsudats de racines colonisées par le *G. etanucatum* ou le *G. monosporum* (Norman et Hooker, 2000). Un impact similaire a été observé quand l'agent pathogène était inoculé dans la mycorhizosphère de fraisiers inoculés avec le *G. etunicatum*. La germination des microconidies du *F. o.* f. sp. *lycopersici* était plus que doublée en présence d'exsudats de plants de tomate colonisés par le *G. mosseae* en comparaison à des exsudats de

plants non-mycorhiziens (Scheffknecht et al., 2006). Des résultats similaires ont été obtenus avec des exsudats de plants non-hôtes de ce champignon pathogène montrant que les modifications de l'exsudation racinaire ne sont pas spécifiques à la tomate (Scheffknecht et al., 2007). Nous avons montré, pour la première fois, que même si les exsudats de plants mycorhiziens peuvent affecter la formation, l'attraction et/ou la germination d'agents pathogènes, de telles modifications n'entraîneraient pas de diminution de la prolifération de cet agent pathogène dans les racines. Bødker et al. (2002) ont suggéré que les CMA n'influenceraient pas le développement de l'agent pathogène *Aphanomyces euteiches* au stage végétatif qui induit la mise en place de nécroses racinaires mais seulement au stade reproductif lorsque les oospores sont produites. En effet, ces auteurs ont observé que l'application du fongicide carbenzamine diminuait le taux de colonisation mycorhizienne de plants de pois et augmentait le pourcentage de racines contenant des oospores de cet agent pathogène. Par contre, le taux de colonisation par les CMA n'était pas corrélé avec la rudesse de la maladie, son incidence et l'activité enzymatique de la glucose-6-phosphate dehydrogenase spécifique de l'agent pathogène.

IIX.6. Les exsudats de racines colonisées par le *G. mosseae* ou le *G. intraradices* ne modifient pas significativement la communauté bactérienne de la rhizosphère de tomate

L'utilisation de la technique de PCR-DGGE à partir du gène 16S codant pour l'ARN ribosomal nous a permis efficacement de caractériser les bactéries prédominantes de la rhizosphère de tomate (cf. chapitre VI). La colonisation de plants de tomate par le *G. mosseae* ou le *G. intraradices* a provoqué une modification significative de la communauté bactérienne de la rhizosphère de ces plants alors que des exsudats de racine de tomate colonisées par ces CMA n'ont pas induit de tels changements. La modification de la communauté bactérienne induite par les CMA (Marschner et Timonen, 2005; Marschner et al., 2001; Wamberg et al., 2003) ne s'effectuerait pas par modification de l'exsudation racinaire comme proposé par de nombreux auteurs (Bansal et Mukerji 1994; Marschner et al., 1997) mais vraisemblablement par d'autres mécanismes. Aussi, les modifications observées de la structure microbienne de la rhizosphère suite à l'inculation du *P. nicotianae* sont faibles et influencent peu les modifications induites par la colonisation mycorhizienne.

Des interactions physiques entre des bactéries et des CMA ont été observées par certains auteurs. La capacité de rhizobium et de pseudomonas d'adhérer aux spores et aux hyphes du *G. margarita* sous des conditions stériles ont été rapportées comme dépendantes de la variété bactérienne (Bianciotto et al., 1996a). Les bactéries adhéreraient aux structures des CMA selon deux étapes. Dans un premier temps, l'attachement serait gouverné par des paramètres physico-chimiques comme de l'attraction électrostatique puis, dans un deuxième temps, renforcé par des composés de

surface des bactéries. La capacité du *Pseudomonas fluorescens* d'adhérer aux structures du *G. intraradices* s'est avérée comme dépendante de la capacité de former des biofilms puisque des mutants des *Azospirillum brasilense* et *Rhizobium leguminosarum* dont les polysaccharides extracellulaires essentiels à la formation de biofilms sont affectés ont perdu l'aptitude d'adhérer aux racines et aux structures mycorhiziennes (Bianciotto et al., 2001a). Aussi, des mutants mucoïdes (présentant une biosynthèse d'alginate activée) adhèrent davantage aux hyphes de ce CMA que la variété sauvage CHAO (Bianciotto et al., 2001b). D'autres auteurs ont également rapporté la propriété de certaines bactéries d'adhérer aux hyphes ou aux spores de CMA qui dépend de l'espèce bactérienne et mycorhizienne et de la vitalité des mycorhizes (Levy et al., 2003; Toljander et al., 2006). Ces bactéries peuvent également exercer une activité saprotrophe qui érode les couches superficielles de spores de CMA (Roesti et al., 2005). Ainsi, la présence de structures fongiques pourrait être essentielle à la compétence des bactéries à se maintenir dans la mycorhizosphère, leur permettant la formation de biofilms ou leur servant de substrat.

Néanmoins, les CMA peuvent avoir des effets négatifs sur la maintenance de certaines bactéries du sol (Bansal et Mukerji 1994; Cavagnaro et al., 2006; Christensen et Jakobsen, 1993; Ravnskov et al., 1999), probablement en raison de compétition pour les nutriments inorganiques.

IIX.7. Les bactéries associées aux spores du *G. mosseae* stérilisées en surface sont antagonistes des agents pathogènes du sol *P. nicotianae*, *Fusarium solani* et du *F. oxysporum, in vitro*

La stérilisation de surface de spores du *G. mosseae* (BEG 12) inoculées sur un milieu de culture standard nous a permis de recueillir 18 isolats bactériens (chapitre VII). Le séquençage du gène 16S de ces bactéries nous a permis de les classer en neuf groupes phylogénétiquement distincts. Ces isolats appartenaient à trois genres différents : *Paenibacillus*, *Bacillus* et *Methylobacterium*. Un total de trente-quatre bandes a été observé suite au PCR-DGGE effectué par amplification du gène ribosomal 16S directement à partir du même type de spores suggérant que des taxons bactériens non-cultivables sur milieu standard sont présents au niveau de ces spores. Les isolats identifiés en tant que *B. simplex*, *B. niacini*, *B. drententis*, *Paenibacillus* spp. et *Bacillus* sp. (à partir du milieu de culture) se sont avérés, *in vitro*, antagonistes envers différents agents pathogènes du sol, surtout vis-à-vis du *P. nicotianae* mais aussi envers le *F. solani* et le *F. oxysporum*. L'antibiotique polymyxine B1 et d'autres composés analogues (antagonistes envers *F. solani* et *F. acuminatum*) ont été identifiés dans les exsudats du *Panibacillus* sp. variété B2 isolée dans la mycorhizosphère du *G. mosseae* (Selim et al., 2005). Cette variété induit de l'antagonisme vis-à-vis du *P. nicotianae*, *in vitro* et *in vivo*, réduit le taux de nécroses racinaires formées par cet agent pathogène, manifeste des activités cellulolytique, protéolitique, chitinolitique et pectinolitique et stimule la colonisation de racines de tomate par le *G. mosseae* (Budi et al., 1999; Budi et al., 2000). D'autres bactéries (spécialement des *Bacillus* et *Paenibacillus*) ont été isolées à partir de structures extraracinaires ou intraracinaires de CMA ou de

l'hyphosphère (zone du sol influencée par le mycélium mycorhizien)(Andrade et al., 1997; Artursson et Jansson, 2003; Filippi et al., 1998; MacDonald et al., 1982; Mansfeld-Giese et al., 2002; Mayo et al., 1986; Secilia et Bagyaraj, 1987; Xavier et Germida, 2003). Grâce à un séquençage du gène 16S, le *Candidatus* Glomeribacter gigasporarum a été identifié comme étant un *Burkholderiaceae* qui forme une endosymbiose obligatoire avec le *Gigaspora margarita* (Bianciotto et Bonfante, 2002; Bianciotto et al., 1996b ; 2000 ; 2003) mais aussi avec d'autres espèces de *Gigasporaceae* (Bonfante, 2003). Les caractéristiques phénotypiques de cette endobactérie qui se transmettrait de façon verticale ont été ensuite décrites plus en détail (Bianciotto et al., 2004; Jargeat et al., 2004). Les bactéries ainsi identifiées par nous-mêmes et d'autres auteurs seraient inoculées en même temps que les CMA et s'établiraient dans la rhizosphère et l'hyphosphère. Ces bactéries pourraient être responsables (au moins partiellement) du biocontrôle que les CMA induisent sur les agents pathogènes du sol. Elles permettraient également d'expliquer les modifications que nous avons observées au niveau des communautés bactériennes au niveau de la rhizosphère de tomate suite à l'inoculation du *G. mosseae* (cf. chapitre VI).

IIX.8. Autres mécanismes de biocontrôle des champignons mycorhiziens à arbuscules sur le *P. nicotianae*, chez la tomate

Par utilisation de profiles d'acides gras, Larsen et Bødker (2001) ont observé la décroissance de la biomasse et des réserves énergétiques du *G. mosseae* et du

Aphanomyces euteiches co-inoculés à des racines de pois. Le *P. nicotianae* et le *G. mosseae* n'occupaient jamais simultanément les mêmes tissus racinaires de tomate (Cordier et al., 1996). La réduction de l'ampleur de la colonisation mycorhizienne par un certain nombre d'agents pathogènes a également été rapportée (Bååth et Hayman, 1983; Davis et Menge, 1980; Krishna et Bagyaraj, 1983). Les CMA pourraient ainsi réduire le développement des agents pathogènes au niveau du sol et des racines par compétition pour les nutriments et les espaces à coloniser.

Le *P. nicotianae* est un agent pathogène du sol qui provoque des nécroses racinaires. Dans des conditions de carence en eau ou en nutriments, la réduction de la biomasse racinaire fonctionnelle entraîne la diminution de la biomasse aérienne et même la mort de la plante en cas de sécheresse intense. Il a été montré que les CMA sont capables de réduire les dommages causés par un stress hydrique (Allen et al., 1981; Augé et Duan, 1991; Levy et Krikun, 1980; Nelsen et Safir, 1982). Ils induiraient notamment une augmentation de la biomasse et des ramifications racinaires (Norman et al., 1996). Ils permettraient ainsi de façon indirecte de contrebalancer les dommages causés par l'agent pathogène. En augmentant l'apport en phosphore inorganique à des plants de tomate inoculés avec le *P. nicotianae*, Trotta et al. (1996) n'ont pas observé une diminution des symptômes de la maladie provoqués par cet agent pathogène montrant que l'augmentation de la nutrition en phosphore n'est pas un mécanisme qui permettrait significativement aux CMA d'induire la mise en place de biocontrôle.

IIX.9. Conclusion générale

Le *G. mosseae* mais aussi le *G. intraradices* permettent la mise en place de biocontrôle chez la tomate infectée par l'agent pathogène du sol le *P. nicotianae* de façon locale (au niveau des tissus colonisés par les CMA), mais aussi de façon systémique (pour ce qui concerne assurément le *G. mosseae*). La stimulation locale et systémique des réactions de défense de la plante s'avère, selon nous, le mécanisme prépondérant permettant la réduction de la prolifération des agents pathogènes et des symptômes qu'ils provoquent. L'implication de l'acide jasmonique dans ces réactions est une voie dont l'étude permettra sûrement d'avancer grandement dans la compréhension des mécanismes qui régissent le biocontrôle. La stimulation des mécanismes de défense ne permet toutefois pas d'expliquer en totalité les caractéristiques du biocontrôle observé. La modification des exsudats racinaires induits par la colonisation mycorhizienne ne permettrait ni de façon directe ni indirecte (par modification de la microflore rhizosphérique) de diminuer la prolifération intraracinaire de cet agent pathogène. Par contre, les bactéries identifiées dans notre étude mais aussi dans d'autres au niveau des structures de CMA tout comme les bactéries en croissance à la surface des structures mycorhiziennes pourraient réduire dans le sol mais aussi dans les racines le développement de l'agent pathogène dont habileté à induire des symptômes serait ainsi réduite. Le rôle de ces bactéries dans le biocontrôle mais aussi dans d'autres effets bénéfiques sur la physiologie de la plante hôte attribués généralement aux CMA est à étudier.

La mycorhizosphère est une zone complexe du sol où chacun des intervenants (racines, champignons mycorhiziens et autres microorganismes du sol) agit sur la

croissance et la physiologie des autres. Les CMA agiraient sur l'équilibre de la mycorhizosphère principalement non pas par modification de l'exsudation racinaire ou par libération d'exsudats mais en constituant un habitat pour certains microorganismes endogènes, en servant de support aux bactéries qui forment ainsi des biofilms ou de substrat aux microorganismes saprotrophes.

Chapitre IX

Bibliographie générale

Abdalla, M.E. et G.M. Abdel-Fattah. 2000. Influence of the endomycorrhizal fungus *Glomus mosseae* on the development of peanut pod rot disease in Egypt. Mycorrhiza 10:29-35.

Addepalli, M.K. et Y. Fujita. 2001. Serological detection of red rot disease initiation stages of microbial pathogen, *Pythium porphyrae* (Oomycota) on *Porphyra yezoensis*. Journal of Applied Phycology 13:221-227.

Akiyama, K., K.-I. Matsuzaki et H. Hayashi. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824-827.

Albertsen, A., S. Ravnskov, H. Green, D.F. Jensen et J. Larsen. 2006. Interactions between the external mycelium of the mycorrhizal fungus *Glomus intraradices* and other soil microorganisms as affected by organic matter. Soil Biology & Biochemistry 38:1008-1014.

Allen, R.N. et F.J. Newhook. 1973. Chemotaxis of zoospores of *Phytophthora cinnamomi* to ethanol in capillaires of soil pore dimensions. Transactions of the British Mycological Society 61:287-302.

Allen, M., W.K. Smith, T.S. Moore et M. Christensen. 1981. Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal *Bouteloua gracilis*. New Phytologist 88:683-693.

Ames, R.N., C.P.P. Reid et E.R. Ingham. 1984. Rhizospheres bacterial population responses to roots colonization by a vesicular-arbuscular mycorrhizal fungus. New Phytolologist 96:555-563.

Amora-Lazcano, E., M.M. Vázquez et R. Azcón. 1998. Response of nitrogen-transforming microorganisms to arbuscular mycorrhizal fungi. Biology & Fertility of Soils 27:65-70.

Andrade, G., K.L. Mihara, R.G. Linderman et G.J. Bethlenfalvay. 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant & Soil 192:71-79.

Andrade, G., K.L. Mihara, R.G. Linderman et G.J. Bethlenfalvay. 1998. Soil aggregation status and rhizobacteria in the mycorrhizosphere. Plant & Soil 202:89-96.

Artursson, V. et J.K. Jansson. 2003. Use of bromodeoxyuridine immunocapture to identify active bacteria associated with arbuscular mycorrhizal hyphae. Applied & Environmental Microbiology 69:6208-6215.

Artursson, V., R.D. Finlay et J.K. Jansson. 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environmental Microbiology 8:1-10.

Atlas, R.M. et R. Bartha. 1997. Microbial ecology: fundamentals and applications Benjamin Cummings Science Publishing, California.

Augé, R.M. et X. Duan. 1991. Mycorrhizal fungi and non-hydraulic root signals of soil drying. Plant Physiology 103:107-116.

Azaizeh, H.A., H. Marschner, V. Romheld et L. Wittenmayer. 1995. Effects of a vesicular-arbuscular mycorrhizal fungus and other soil microorganisms on growth, mineral nutrient acquisition and root exudation of soil-grown maize plants. Mycorrhiza 5:321-327.

Azcón-Aguilar, C. et J.M. Barea. 1992. Interactions between mycorrhizal fungi and other rhizosphere microorganisms. Dans *Mycorrhizal Functioning* (ed. M. F. Allen), pp. 163-198. London: Chapman & Hall.

Azcón-Aguilar, C. et J.M. Barea. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens - An overview of the mechanisms involved. Mycorrhiza 6:457-464.

Azcón, R., M. Gomez et R. Tobar. 1996. Physiological and nutritional responses by *Lactuca sativa* to nitrogen sources and mycorrhizal fungi under drought conditions. Biology & Fertility of Soils 22:156-161.

Bååth, E. et D.S. Hayman. 1983. Plant growth responses to vesicular-arbuscular mycorrhizae XIV. Interactions with Verticillium wilt on tomato plants. New Phytologist 95:419-426.

Bååth, E., L.O. Nilsson, H. Göransson et H. Wallander. 2004. Can the extent of degradation of soil fungal mycelium during soil incubation be used to estimate ectomycorrhizal biomass in soil? Soil Biology & Biochemistry 36:2105-2109.

Bago, B., H. Vierheilig, Y. Piché et C. Azcón-Aguilar. 1996. Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal

fungus *Glomus intraradices* grown in monoxenic culture. New Phytologist 133:273-280.

Bago, B., P.E. Pfeffer, D.D. Douds, J. Brouillette, G. Bécard et Y. Shachar-Hill. 1999. Carbon metabolism in spores of the arbuscular mycorrhizal fungus *Glomus intraradices* as revealed by nuclear magnetic resonance spectroscopy. Plant Physiology 121:263-271.

Bago, B., P.E. Pfeffer et Y. Shachar-Hill. 2000. Carbon metabolism and transport in arbuscular mycorrhizas. Plant Physiology 124:949-957.

Bagyaraj, D.J. et J.A. Menge. 1978. Interactions between a VA mycorrhiza and Azobacter and their effects on rhizosphere microflora and plant growth. New Phytologist. 80:567-573.

Bais, H.P., S.W. Park, T.L. Weir, R.M. Callaway et J.M. Vivanco. 2004. How plants communicate using the underground information superhighway. Trends in Plant Science 9:26-32.

Bais, H.P., T.L. Weir, L.G. Perry, S. Gilroy et J.M. Vivanco. 2006. The role of root exudates in rhizosphere interations with plants and other organisms. Annual Review of Plant Biology 57:233-266.

Baker, K.F. et R.J. Cook. 1974. Biological control of plant pathogens. (ed. W.H. Freeman). San Francisco, California.

Balaji, B., M.J. Poulain, H. Vierheilig et Y. Piché. 1995. Reponses of an arbuscular mycorrhizal fungus, *Gisgaspora margarita*, to exudates and volatiles from the Ri T-DNA-transformed roots of nonmycorrhizal and mycorrhizal mutants of *Pisum sativum* L Sparkle. Experimental Mycology 19:275-283.

Bansal, M. et K.G. Mukerji 1994. Positive correlation between VAM-induced changes in root exudation and mycorrhizosphere mycoflora. Mycorrhiza 5:39-44.

Barea, J.M., R. Azcón et C. Azcón-Aguilar. 1983. Interactions between phosphate solubilizing bacteria and VA mycorrhiza to improve plant utilization of rock phosphate in non acidic soils. Dans *3rd International Congress on Phosphorus Compounds*, pp. 127–144. Brussels:October 4-6.

Barea, J. M., C. Azcón-Aguilar, and R. Azcón. 1997. Interactions between mycorrhizal fungi and rhizosphere microorganisms within the context of sustainable soil-plant

systems. In *Multitrophic interactions in terrestrial systems*, (ed. A. C. Gange et V. K. Brown), pp. 65-77. Osney Mead: Blackwell Science Publ.

Barea, J.M., M. Toro, M.O. Orozco, E. Campos et R. Azcón. 2002. The application of isotopic (P-32 and N-15) dilution techniques to evaluate the interactive effect of phosphate-solubilizing rhizobacteria, mycorrhizal fungi and Rhizobium to improve the agronomic efficiency of rock phosphate for legume crops. Nutrient Cycling in Agroecosystems 63:35-42.

Barea, J.M., M.J. Pozo, R. Azcón et C. Azcón-Aguilar. 2005. Microbial co-operation in the rhizosphere. Journal of Experimental Botany 56:1761-1778.

Barroso, C.B. et E. Nahas. 2005. The status of soil phosphate fractions and the ability of fungi to dissolve hardly soluble phosphates. Applied Soil Ecology 29:73-83.

Baudoin, E., E. Benizri et A. Guckert. 2003. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biology & Biochemistry 35:1183-1192.

Beatty, P.H. et S.E. Jensen. 2002. *Paenibacillus polymyxa* produces fusaricidin-type antifungal antibiotics active against *Leptosphaeria maculans*, the causative agent of blackleg disease of canola. Canadian Journal of Microbiology 48:159-169.

Bécard, G. et J.A. Fortin. 1988. Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. New Phytolologist 108:211-218.

Bécard, G. et Y. Piché. 1989. Fungal growth stimulation by CO_2 and root exudates in vesicular-arbuscular mycorrhizal symbiosis. Applied & Environmental Microbiology 55:2320-2325.

Bécard, G., L.P. Taylor, D.D. Douds, P.E. Pfeffer et L.W. Doner. 1995. Flavonoids are not necessary plant signal compounds in arbuscular mycorrhizal symbioses. Molecular Plant-Microbe Interactions 8:252-258.

Bécard, G., S. Kosuta, M. Tamasloukht, N. Séjalon-Delmas et C. Roux. 2004. Partner communication in the arbuscular mycorrhizal interaction. Canadian Journal of Botany 82:1186-1197.

Bel Rhlid, R., S. Chabot, Y. Piché et R. Chênevert. 1993. Isolation and identification of flavonoids from Ri T-DNA-tranformed roots (*Daucus carota*) and their significance in vesicular-arbuscular mycorrhiza. Phytochemistry 33:1369-1371.

Benhamou, N., J.A. Fortin, C. Hamel, M. St-Arnaud et A. Shatilla. 1994. Resistance responses of mycorrhizal Ri T-DNA-transformed carrot roots to infection by *Fusarium oxysporum* f. sp. *chrysanthemi*. Phytopathology 84:958-968.

Berta, G., A. Fusconi, S. Sampò, G. Lingua, S. Perticone et O. Repetto. 2000. Polyploidy in tomato roots as affected by arbuscular mycorrhizal colonization. Plant & Soil 226:37-44.

Berta, G., S. Sampo, E. Gamalero, N. Massa et P. Lemanceau. 2005. Suppression of *Rhizoctonia* root-rot of tomato by *Glomus mossae* BEG12 and *Pseudomonas fluorescens* A6RI is associated with their effect on the pathogen growth and on the root morphogenesis. European Journal of Plant Pathology 111:279-288.

Bertin, C., X. Yang et L.A. Weston. 2003. The role of root exudates and allelochemicals in the rhizosphere. Pland & Soil 256:67-83.

Bezzate, S., S. Aymerich, R. Chambert, S. Czarnes, O. Berge et T. Heulin. 2000. Disruption of the *Paenibacillus polymyxa* levansucrase gene impairs its ability to aggregate soil in the wheat rhizosphere. Environmental Microbiology 2:333-342.

Bianciotto, V., D. Minerdi, S. Perotto et P. Bonfante. 1996a. Cellular interactions between arbuscular mycorrhizal fungi and rhizosphere bacteria. Protoplasma 193:123-131.

Bianciotto, V., C. Bandi, D. Minerdi, M. Sironi, H.V. Tichy et P. Bonfante. 1996b. An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. Applied & Environmental Microbiology 62:3005-3010.

Bianciotto, V., E. Lumini, L. Lanfranco, D. Minerdi, P. Bonfante et S. Perotto. 2000. Detection and identification of bacterial endosymbionts in arbuscular mycorrhizal fungi belonging to the family Gigasporaceae. Applied & Environmental Microbiology 66:4503-4509.

Bianciotto, V., S. Andreotti, R. Balestrini, P. Bonfante et S. Perotto. 2001a. Extracellular polysaccharides are involved in the attachment of *Azospirillum brasilense* and *Rhizobium leguminosarum* to arbuscular mycorrhizal structures. European Journal of Histochemistry 45:39-49.

Bianciotto, V., S. Andreotti, R. Balestrini, P. Bonfante et S. Perotto. 2001b. Mucoid mutants of the biocontrol strain *Pseudomonas fluorescens* CHA0 show increased

ability in biofilm formation on mycorrhizal and nonmycorrhizal carrot roots. Molecular Plant-Microbe Interactions 14:255-260.

Bianciotto, V. et P. Bonfante. 2002. Arbuscular mycorrhizal fungi: a specialised niche for rhizospheric and endocellular bacteria. Antonie van Leeuwenhoek International - Journal of General & Molecular Microbiology 81:365-371.

Bianciotto, V., S. Perotto, J.M. Ruis-Lorano et P. Bonfante. 2002. Arbuscular mycorrhizal fungi and soil bacteria: from cellular investigations to biotechnological perspectives. Dans *Mycorrhizal Technology in Agriculture*: from genes to bioproducts (ed. S. Gianinazzi , H. Schuepp, J. M. Barea and K. Haselwandter), pp. 19-31.

Bianciotto, V., E. Lumini, P. Bonfante et P. Vandamme. 2003. '*Candidatus* Glomeribacter gigasporarum' gen. nov., sp. nov., an endosymbiont of arbuscular mycorrhizal fungi. International Journal of Systematic and Evolutionary Microbiology 53:121-124.

Bianciotto, V., A. Genre, P. Jargeat, E. Lumini, G. Bécard et P. Bonfante. 2004. Vertical transmission of endobacteria in the arbuscular mycorrhizal fungus *Gigaspora margarita* through generation of vegetative spores. Applied & Environmental Microbiology 70:3600-3608.

Bieleski, R.L. 1973. Phosphate pools, phosphate transport and phosphate availability. Annual Review of Plant Physiology 24:225-252.

Bircher, U. et H.R. Hohl. 1997a. Environmental signalling during induction of appressorium formation in *Phytophthora*. Mycological Research 101:395-402.

Bircher, U. et H.R. Hohl. 1997b. Surface glycoproteins associated with appressorium formation and adhesion in *Phytophthora palmivora*. Mycological Research 101:769-775.

Bircher, U. et H.R. Hohl. 1999. A role for calcium in appressorium induction in *Phytophthora palmivora*. Botanica Helvetica 109:55-65.

Blancard, D. 1988. Maladies de la tomate: observer, identifier, lutter. Paris: INRA Eds.

Bødker, L., R. Kjøller, K. Kristensen et S. Rosendahl. 2002. Interactions between indigenous arbuscular mycorrhizal fungi and *Aphanomyces euteiches* in field-grown pea. Mycorrhiza 12:7-12.

Boer, W.D., L.B. Folman, R.C. Summerbell et L. Boddy. 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29:795-811.

Böhme, L., U. Langer et F. Böhme. 2005. Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. Agriculture Ecosystems & Environment 109:141-152.

Bonfante, P. 2003. Plants, mycorrhizal fungi and endobacteria: a dialog among cells and genomes. Biological Bulletin 204:215-220.

Borowicz, V.A. 2001. Do arbuscular mycorrhizal fungi alter plant-pathogen relations? Ecology 82: 3057-3068.

Bowen, G.D. et A.D. Rovira. 1999. The rhizosphere and its management to improve plant growth. Advances in Agronomy 66:1-102.

Brandao, P.F.B., M. Torimura, R. Kurane et A.T. Bull. 2002. Dereplication for biotechnology screening: PyMS analysis and PCR-RFLP-SSCP (PRS) profiling of 16S rRNA genes of marine and terrestrial actinomycetes. Applied Microbiology & Biotechnology 58:77-83.

Brimner, T.A. et G.J. Boland. 2003. A review of the non-target effects of fungi used to biologically control plant diseases. Agriculture Ecosystems & Environment 100:3-16.

Brodie, E., S. Edwards et N. Clipson. 2003. Soil fungal community structure in a temperate upland grassland soil. FEMS Microbiology Ecology 45:105-114.

Bruehl, G.W. 1987. Soilborne plant pathogens. Macmillan Publishing Co, New York.

Budi, S.W., D. van Tuinen, G. Martinotti et S. Gianinazzi. 1999. Isolation from the *Sorghum bicolor* mycorrhizosphere of a bacterium compatible with arbuscular mycorrhiza development and antagonistic towards soilborne fungal pathogens. Applied & Environmental Microbiology 65:5148-5150.

Budi, S.W., D. van Tuinen, C. Arnould, E. Dumas-Gaudot, V. Gianinazzi-Pearson et S. Gianinazzi. 2000. Hydrolytic enzyme activity of *Paenibacillus* sp. strain B2 and effects of the antagonistic bacterium on cell integrity of two soil-borne pathogenic fungi. Applied Soil Ecology 15:191-199.

Cahill, D.M. et A.R. Hardham. 1994. Exploitation of zoospore taxis in the development of a novel dipstick immunoassay for the specific detection of *Phytophthora cinnamomi*. Phytopathology 84:193-200.

Calvet, C., J.M. Barea et J. Pera. 1992. *In vitro* interactions between the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* and some saprophytic fungi isolated from organic substrates. Soil Biology and Biochemistry 24:775-780.

Calvo-Bado, L.A., G. Petch, N.R. Parsons, J.A.W. Morgan, T.R. Pettitt et J.M. Whipps. 2006. Microbial community responses associated with the development of oomycete plant pathogens on tomato roots in soilless growing systems. Journal of Applied Microbiology 100:1194-1207.

Campbell, C.D., S.J. Grayston et D.J. Hirst. 1997. Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. Journal of Microbiological Methods 30:33-41.

Campbell, C.A., V.O. Biederbeck, G. Wen, R.P. Zentner, J. Schoenau et D. Hahn. 1999. Seasonal trends in soil biochemical attributes: effects of crop rotation in the semiarid prairie. Canadian Journal of Soil Science 79:73-84.

Camprubí, A., C. Calvet et V. Estaun. 1995. Growth enhancement of *Citrus reshni* after inoculation with *Glomus intraradices* and *Trichoderma aureoviride* and associated effects on microbial populations and enzyme activity in potting mixes. Plant & Soil 173:233-238.

Carlile, M.J. 1983. Motility, taxis and tropism. Dans *Phytophthora: its biology, taxonomy, ecology, and pathology* (ed. Erwin, D., Bartnicki-Garcia, S. et Tsao), pp 95-107. St-Paul, Minnesota: The American Phytopathological Society Press.

Caron, M., J.A. Fortin et C. Richard. 1985. Influence of substrate on the interaction of *Glomus intraradices* and *Fusarium oxysporum* f. sp. r*adicis-lycopersici* on tomatoes. Plant & Soil 87:233-239.

Caron, M., J.A. Fortin et C. Richard. 1986a. Effect of *Glomus intraradices* on infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomatoes over a twelve-week period. Canadian Journal of Botany 64:552-556.

Caron, M., J.A. Fortin et C. Richard. 1986b. Effect of inoculation sequence on the interaction between *Glomus intraradices* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomatoes. Canadian Journal of Plant Pathology 8:12-16.

Caron, M., J.A. Fortin et C. Richard. 1986c. Effect of phosphorus concentration and *Glomus intraradices* on Fusarium root rot of tomatoes. Phytopathology 76:942-946.

Caron, M., C. Richard et J.A. Fortin. 1986d. Effect of preinfestation of the soil by a vesicular-arbuscular mycorrhizal fungus, *Glomus intraradices*, on Fusarium crown and root rot of tomatoes. Phytoprotection 67:15-19.

Catford, J.G., C. Staehelin, S. Lerat, Y. Piché et H. Vierheilig. 2003. Suppression of arbuscular mycorrhizal colonization and nodulation in split-root systems of alfalfa after pre-inoculation and treatment with Nod factors. Journal of Experimental Botany 54:1481-1487.

Cavagnaro, T.R., L.E. Jackson, J. Six, H. Ferris, S. Goyal, D. Asami et K.M. Scow. 2006. Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. Plant & Soil 282:209-225.

Chabot, S., G. Bécard et Y. Piché. 1992. Life cycle of *Glomus intraradix* in root organ culture. Mycologia 8:315-321.

Chin, A.W.T.F.C., G.V. Bloemberg, I.H.M. Mulders, L.C. Dekkers et B.J.J. Lugtenberg. 2000. Root colonization by phenazine-1-carboxamide-producing bacterium *Pseudomonas chlororaphis* PCL1391 is essential for biocontrol of tomato foot and root rot. Molecular Plant-Microbe Interactions 13:1340-1345.

Christensen, H. et I. Jakobsen. 1993. Reduction of bacterial growth by a vesicular-arbuscular mycorrhizal fungus in the rhizosphere of cucumber (*Cucumis sativus* L). Biology & Fertility of Soils 15:253-258.

Chung, Y.R., C.H. Kim, I. Hwang et J. Chun. 2000. *Paenibacillus koreensis* sp. nov., a new species that produces an iturin-like antifungal compound. International Journal of Systematic & Evolutionary Microbiology 50:1495-1500.

Citernesi, A.S., P. Fortuna, G. Bagnoli et M. Giovanetti. 1996. The occurence of antagonostic bacteria in *Glomus mosseae* pot cultures. Agronomie 16:671-677.

Cliquet, J.B. et G.R. Stewart. 1993. Ammonia assimilation in *Zea mays* L. infected with a vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum*. Plant Physiology 101:865-871.

Connolly, M.S., N. Williams, C.A. Heckman et P.F. Morris. 1999. Soybean isoflavones trigger a calcium influx in *Phytophthora sojae*. Fungal Genetics & Biology 28:6-11.

Cook, R. et K. Baker. 1983. The nature and practice of biological control of plant pathogens. St-Paul, Minnesota: The American Phytopathological Society Press.

Cordier, C., S. Gianinazzi et V. Gianinazzi-Pearson. 1996. Colonisation patterns of root tissues by *Phytophthora nicotianae* var. *parasitica* related to reduced disease in mycorrhizal tomato. Plant & Soil 185:223-232.

Cordier, C., M.J. Pozo, J.M. Barea, S. Gianinazzi et V. Gianinazzi-Pearson. 1998. Cell defense responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. Molecular Plant-Microbe Interactions 11:1017-1028.

Corgié, S.C., F. Fons, T. Beguiristain et C. Leyval. 2006. Biodegradation of phenanthrene, spatial distribution of bacterial populations and dioxygenase expression in the mycorrhizosphere of *Lolium perenne* inoculated with *Glomus mosseae*. Mycorrhiza 16:207-212.

Curl, E.A. et B. Truelove. 1986. The rhizosphere. New York: Springer-Verlag.

D'arcy, A.L. 1982. Etude des exsudats racinaires de Soja et de Lentille. I. Cinétique d'exsudation des composés phénoliques, des amino acides et des sucres, au cours des premiers jours de la vie des plantules. Plant & Soil 68:399-403.

da Silva, G.A., E. Lumini, L. Costa Maia, P. Bonfante et V. Bianciotto. 2006. Phylogenetic analysis of Glomeromycota by partial LSU rDNA sequences. Mycorrhiza 16:183-189.

Dalpé, Y. 2003. Mycorrhizal fungi biodiversity in Canadian soils. Canadian Journal of Soil Science 83:321-330.

Dar, G.H., M.Y. Zargar et G.M. Beigh. 1997. Biocontrol of fusarium root rot in the common bean (*Phaseolus vulgaris* L) by using symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*. Microbial Ecology 34:74-80.

Darrah, P.R. 1996. Rhizodeposition under ambient and elevated CO_2 Levels. Plant & Soil 187:265-275.

Davis, R.M. et J.A. Menge. 1980. Influence of *Glomus fasciculatus* and soil phosphorus on Phytophthora root rot of Citrus. Phytopathology 70: 447-452.

Deacon, J.W. 1996. Ecological implications of recognition events in the pre-infection stages of root pathogens. New Phytologist 133:135-145.

Deacon, J.W. et S.P. Donaldson. 1993. Molecular recognition in the homing responses of zoosporic fungi, with special reference to *Pythium* and *Phytophthora.* Mycological Research 97:1153-1171.

Deacon, J.W. et G. Saxena. 1998. Germination triggers of zoospore cysts of *Aphanomyces euteiches* and *Phytophthora parasitica.* Mycological Research 102:33-41.

Dehne, H.-W. 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72:1115-1119.

deSouza, F.A., G.A. Kowalchuk, P. Leeflang, J.A. vanVeen et E. Smit. 2004. PCR-denaturing gradient gel electrophoresis profiling of inter- and intraspecies 18S rRNA gene sequence heterogeneity is an accurate and sensitive method to assess species diversity of arbuscular mycorrhizal fungi of the genus *Gigaspora.* Applied & Environmental Microbiology 70:1413-1424.

Diedhiou, P.M., J. Hallmann, E.C. Oerke et H.W. Dehne. 2003. Effects of arbuscular mycorrhizal fungi and a non-pathogenic *Fusarium oxysporum* on *Meloidogyne incognita* infestation of tomato. Mycorrhiza 13:199-204.

Dijksterhuis, J., M. Sanders, L.G.M. Gorris et E.J. Smid. 1999. Antibiosis plays a role in the context of direct interaction during antagonism of *Paenibacillus polymyxa* towards *Fusarium oxysporum.* Journal of Applied Microbiology 86:13-21.

Diop, T.A., G. Bécard et Y. Piché. 1992. Long-term *in vitro* culture of an endomycorrhizal fungus, *Gigaspora margarita,* on Ri T-DNA transformed roots of carrot. Symbiosis 12:249-259.

Diouf, D., R. Duponnois, A.T. Ba, M. Neyra et D. Lesueur. 2005. Symbiosis of *Acacia auriculiformis* and *Acacia mangium* with mycorrhizal fungi and *Bradyrhizobium*

spp. improves salt tolerance in greenhouse conditions. Functional Plant Biology 32:1143-1152.

Djordjevic, M.A. 1987. Clovers secrete specific phenolics compounds which either stimulate or repress Nod gene expression in *Rhizobium trifolii*. The EMBO Journal 6:1173-1179.

Dodd, J.C. 2000. The role of arbuscular mycorrhizal fungi in agro- and natural ecosystems. Outlook on Agriculture 29:55-62.

Domsch, K.H., W. Gams et T.H. Anderson. 1980. Compendium of soil fungi Academic Press, London.

Donaldson, S.P. et J.W. Deacon. 1993a. Effects of amino acids and sugars on zoospore taxis, encystment and cyst germination in *Pythium aphanidermatum* (Edson) Fitzp., *P. catenulatum* Matthews and *P. dissotocum* Drechs. New Phytologist 123:289-295.

Donaldson, S.P. et J.W. Deacon. 1993b. Differential encystment of zoospores of Pythium species by saccharides in relation to establisment on roots. Physiological & Molecular Plant Pathology 42:177-184.

Doner, L.W. et G. Bécard. 1991. Solubilization of gellan gels by chelation of cations. Biotechnological Technology 5:25-28.

Duineveld, B.M., G.A. Kowalchuk, A. Keijzer, J.D. van Elsas et J.A.V. Veen. 2001. Analysis of bacterial communities in the rhizosphere of Chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. Applied & Environmental Microbiology 67:172-178.

Dukes, P.D. et J.L. Apple. 1961. Chemotaxis of zoospores of *Phytopthora parasitica* var. *nicotianae* by plant roots and certain chemical solutions. Phytopathological Notes 51:195-197.

Dunbar, J., S. Takala, S.M. Barns, J.A. Davis et C.R. Kuske. 1999. Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. Applied & Environmental Microbiology 65:1662-1669.

Edwards, S.G., J.P.W. Young et A.H. Fitter. 1989. Isolation and direct complete nucleotide determination of entire gene. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Research 17:7843-53.

Edwards, S.G., J.P.W. Young et A.H. Fitter. 1998. Interactions between *Pseudomonas fluorescens* biocontrol agents and *Glomus mosseae*, an arbuscular mycorrhizal fungus, within the rhizosphere. FEMS Microbiology Letters 166:297-303.

Elsen, A., S. Declerck et D. De Waele. 2001. Effects of *Glomus intraradices* on the reproduction of the burrowing nematode (*Radopholus similis*) in dixenic culture. Mycorrhiza 11: 49-51.

Erwin, C., S. Bartnicki-Garcia et P.H. Tsao. 1983. Phytophthora: its biology, taxonomy, ecology and pathology. St-Paul, Minnesota: The American Phytopathological Society Press.

Erwin, C. et O.K. Ribeiro. 1996. Phytophthora diseases worlwide. St-Paul, Minnesota: The American Phytopathological Society Press.

Estrada-Garcia, M.T., T.C. Ray, J.R. Green, J.A. Callow et J.F. Kennedy. 1990. Encystment of *Pythium aphanidermatum* zoospores is induced by root mucilage polysaccharides, pectin and a monoclonal antibody to a surface antigen. Journal of Experimental Botany 41:693-699.

Ezawa, T., T.R. Cavagnaro, S.E. Smith, F.A. Smith et R. Ohtomo. 2004. Rapid accumulation of polyphosphate in extraradical hyphae of an arbuscular mycorrhizal fungus as revealed by histochemistry and a polyphosphate kinase/luciferase system. New Phytologist 161:387-392.

Fabro, G., I. Kovács, V. Pavet, L. Szabados et M.E. Alvarez. 2004. Proline accumulation and *AtP5CS2* gene activation are induced by plant-pathogen incompatible interactions in *Arabidopsis*. Molecular Plant-Microbe Interactions 17:343-350.

Felske, A.D.M., V. Tzeneva, J. Heyrman, M.A. Langeveld, A.D.L. Akkermans et P. De Vos. 2004. Isolation and biodiversity of hitherto undescribed soil bacteria related to *Bacillus niacini*. Microbial Ecology 48:111-119.

Fester, T., W. Maier et D. Strack. 1999. Accumulation of secondary compounds in barley and wheat roots in response to inoculation with an arbuscular mycorrhizal fungus and co-inoculation with rhizosphere bacteria. Mycorrhiza 8:241-246.

Fester, T., B. Hause, D. Schmidt, K. Halfmann, J. Schmidt, V. Wray, G. Hanse et D. Strack. 2002a. Occurrence and localization of apocarotenoids in arbuscular mycorrhizal plant roots. Plant & Cell Physiology 43:256-265.

Fester, T., D. Schmidt, S. Lohse, M.H. Walter, G. Giuliano, P.M. Bramley, P.D. Fraser, B. Hause et D. Strack. 2002b. Stimulation of carotenoid metabolism in arbuscular mycorrhizal roots. Planta 216:148-154.

Fester, T., V. Wray, M. Nimtz et D. Strack. 2005. Is stimulation of carotenoid biosynthesis in arbuscular mycorrhizal roots a general phenomenon? Phytochemistry 66:1781-1786.

Filion, M., M. St-Arnaud et J.A. Fortin. 1999. Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. New Phytologist 141:525-533.

Filion, M., M. St-Arnaud et S.H. Jabaji-Hare. 2003a. Direct quantification of fungal DNA from soil substrate using real-time PCR. Journal of Microbiological Methods 53:67-76.

Filion, M., M. St-Arnaud et S.H. Jabaji-Hare. 2003b. Quantification of *Fusarium solani* f. sp. *phaseoli* in mycorrhizal bean plants and surrounding mycorrhizosphere soil using real-time polymerase chain reaction and direct isolations on selective media. Phytopathology 93:229-235.

Filippi, C., G. Bagnoli, A.S. Citernesi et M. Giovannetti. 1998. Ultrastructural spatial distribution of bacteria associated with sporocarps of *Glomus mosseae*. Symbiosis 24:1-12.

Fitze, D., A. Wiepning, M. Kaldorf et J. Ludwig-Müller. 2005. Auxins in the development of an arbuscular mycorrhizal symbiosis in maize. Journal of Plant Physiology 162:1210-1219.

Fortin, J.A., G. Bécard, S. Declerck, Y. Dalpé, M. St-Arnaud, A.P. Coughlan et Y. Piché. 2002. Arbuscular mycorrhiza on root-organ cultures. Canadian Journal of Botany 80:1-20.

Fracchia, S., M.T. Mujica, I. García-Romera, García-Garrido, M. J. M., J., J.A. Ocampo et A. Godeas. 1998. Interactions between *Glomus mosseae* and arbuscular mycorrhizal sporocarp-associated saprophytic fungi. Plant & Soil 200:131-137.

Fusconi, A., S. Sampò, A. Tahiri-Alaoui, G. Lingua, E. Gnavi et G. Berta. 1997. Ploidy in tomato roots and its variation inuced by *Glomus mosseae* and *Phytophthora nicotianae* var. *parasitica*. Atti Accademia dei Fisiocritici di Siena 15:55-58.

Gagnon, H., J. Grandmaison et R.K. Ibrahim. 1995. Phytochemical and immunocytochemical evidence for the accumulation of 2'-hydroxylupalbigenin in lupin nodules and bacteroids. Molecular Plant-Microbe Interactions 8:131-137.

Galli, U. H. Schüepp, et C. Brunold. 1994. Heavy metal binding by mycorrhizal fungi. Physiologia Plantarum 92:364-368.

Gamalero, E., A. Trotta, N. Massa, A. Copetta, M.G. Martinotti et G. Berta. 2004. Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition. Mycorrhiza 14: 185-192.

Gange, A.C. et H.E. Nice. 1997. Performance of the thistle gall fly, *Urophora cardui*, in relation to host plant nitrogen and mycorrhizal colonization. New Phytologist 137:335-343.

Gange, A.C. 2001. Species-specific responses of a root- and shoot-feeding insect to arbuscular mycorrhizal colonization of its host plant. New Phytologist 150:611-618.

Garbaye, J. 1991. Biological interactions in the mycorrhizosphere. Experientia 47:370-375.

Garbeva, P., L.S. van Overbeek, J.W.L. van Vuurde et J.D. van Elsas. 2001. Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. Microbial Ecology 41:369-383.

García-Garrido, J.M. et J.A. Ocampo. 1988. Interaction between *Glomus mosseae* and *Erwinia carotovora* and its effects on the growth of tomato plants. New Phytologist 110:551-555.

García-Garrido, J.M. et J.A. Ocampo. 1989. Effect of VA mycorrhizal infection of tomato on damage caused by *Pseudomonas syringae*. Soil Biology & Biochemistry 21:165-167.

García-Garrido, J.M. et J.A. Ocampo. 2002. Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. Journal of Experimental Botany 53:1377-1386.

García-Romera, I., J.M. García-Garrido, J. Martín, S. Fracchia, M.T. Mujica, A. Godeas et J.A. Ocampo. 1998. Interactions between saprotrophic *Fusarium* strains and arbuscular mycorrhizas of soybean plants. Symbiosis 24:235-245.

Geelhoed, J.S., W.H. Van Riemsdijk et G.R. Findenegg. 1999. Simulation of the effect of citrate exudation from roots on the plant availability of phosphate adsorbed on goethite. European Journal of Soil Science 50:379-390.

George, E., H. Marschner et I. Jakobsen. 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. Critical Reviews in Biotechnology 15:257-270.

Gerdemann, J. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annual Review of Phytopathology 6:397-418.

Gerdemann, J.W. 1975. Vesicular-arbuscular mycorrhizae. Dans *The development and function of roots* (ed. J. G. Torrey and D. T. Clarkson), pp. 575-591. Academic Press, New-York.

Gianinazzi-Pearson, V., S.E. Smith, S. Gianinazzi et F.A. Smith. 1991. Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhizas. Is H+-ATPase a component of ATP-hydrolysing enzyme activities in plant-fungus interfaces? New Phytologist 117:61-76.

Gianinazzi-Pearson, V., E. Dumas-Gaudot, A. Gollotte, A. Tahiri Alaoui et S. Gianinazzi. 1996. Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. New Phytologist 133:45-57.

Giannakou, I.O., D.G. Karpouzas et D. Prophetou-Athanasiadou. 2004. A novel non-chemical nematicide for the control of root-knot nematodes. Applied Soil Ecology 26:69-79.

Gildon, A. et P.B. Tinker. 1981. A heavy metal-tolerant tolerant strain of a mycorrhizal fungus. Transactions of the British Mycological Society 77:648-649.

Giovannetti, M. et M. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular infection in roots. New Phytologist 84:589-600.

Giovannetti, M., L. Avio, C. Sbrana et A.S. Citernesi. 1993. Factors affecting appressorium development in the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe. New Phytologist 123:115-122.

Glick, B.R. 1995. The enhancement of plant growth promotion by free living bacteria. Canadian Journal of Botany 41:109-117.

Godeas, A., S. Fracchia, M.T. Mujica et J.A. Ocampo. 1999. Influence of soil impoverishment on the interaction between *Glomus mosseae* and saprobe fungi. Mycorrhiza 9:185-189.

Govindarajulu, M., P.E. Pfeffer, H. Jin, J. Abubaker, D.D. Douds, J.W. Allen, H. Bücking, P.J. Lammers et Y. Shachar-Hill. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819-823.

Graham, J., R. Leonard et J. Menge. 1981. Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. Plant Physiology 68:548-552.

Graham, J.H. et R.T. Leonard. 1982. Interaction of light intensity and soil temperature with phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. New Phytologist 91:683-690.

Graham, J.H. 1988. Interaction of mycorrhizal fungi with soilborne plant pathogens and other organisms: an introduction. Phytopathology 78:365-366.

Grandmaison, J., G.M. Olah, M.R. Van Calsteren et V. Furlan. 1993. Characterisation and localization of plant phenolics likely involved in the pathogen resistance expressed by endomycorrhizal roots. Mycorrhiza 3:155-164.

Grayston, S.J., D. Vaughan et D. Jones. 1997. Rhizophere carbon flow in trees, in comparasion with annual plants - The importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5:29-56.

Grayston, S.J., S. Wang, C.D. Campbell et A.C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology & Biochemistry 30:369-378.

Green, H., J. Larsen, P.A. Olsson, D.F. Jensen et I. Jakobsen. 1999. Suppression of the biocontrol agent *Trichoderma harzianum* by mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* in root-free soil. Applied & Environmental Microbiology 65:1428-1434.

Griffiths, B.S., K. Ritz, N. Ebblewhite et G. Dobson. 1999. Soil microbial community structure: Effects of substrate loading rates. Soil Biology & Biochemistry 31:145-153.

Grote, D. et W. Claussen. 2001. Severity of root rot on tomato plants caused by *Phytophthora nicotianae* under nutrient- and light-stress conditions. Plant Pathology 50:702-707.

Gryndler, M. et H. Hrselová. 1998. Effect of diazotrophic bacteria isolated from a mycelium of arbuscular mycorrhizal fungi on colonization of maize roots by *Glomus fistulosum*. Biologia Plantarum 41:617-621.

Gryndler, M., M. Vosátka, H. Hrselová, V. Catská, I. Chvátalová et J. Jansa. 2002. Effect of dual inoculation with arbuscular mycorrhizal fungi and bacteria on growth and mineral nutrition of strawberry. Journal of Plant Nutrition 25:1341-1358.

Halsall, D.M. 1975. Zoospore chemotaxis in Australian isolates of Phytophthora. Canadian Journal of Microbiology 22:409-422.

Hamel, C. 2004. Impact of arbuscular mycorrhizal fungi on N and P cycling in the root zone. Canadian Journal of Soil Science 84:383-395.

Hamel, C. 2007. Extraradical arbuscular mycorrhizal mycelia: shadowy figures in the soil. Dans *Mycorrhizae in crop production* (ed. C. Hamel and C. Plenchette), pp. 1-36. Haworth Press, New York.

Hamel, C., C. Neeser, U. Barrantes-Cartín et D.L. Smith. 1991. Endomycorrhizal fungal species mediate 15N-transfer from soybean to corn in non-fumigated soil. Plant & Soil 138:41-47.

Hamel, C., V. Vujanovic, R. Jeannotte, A. Nakano-Hylander et M. St-Arnaud. 2005a. Negative feedback on a perennial crop: Fusarium crown and root rot of asparagus is related to changes in soil microbial community structure. Plant & Soil 268:75-87.

Hamel, C., V. Vujanovic, A. Nakano-Hylander, R. Jeannotte et M. St-Arnaud. 2005b. Factors associated with Fusarium crown and root rot of asparagus outbreaks in Quebec. Phytopathology 95: 867-873.

Hanson, R.S. et T.E. Hanson. 1996. Methanotrophic bacteria. Microbiological Reviews 60:439 ff.

Hardham, A.R., D.M. Cahill, M. Cope, B.K. Gabor, F. Gubler et G.J. Hyde. 1994. Cell surface antigens of *Phytophthora* spores - biological and taxonomic characterization. Protoplasma 181:213-232.

Hare, P.D., W.A. Cress et J. van Staden. 1999. Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. Journal of Experimental Botany 50:413-434.

Harley, J.L. et E.L. Harley. 1987. A check-list of mycorrhizal in the british flora. New Phytologist 105:1-102.

Harms, G., A.C. Layton, H.M. Dionisi, I.R. Gregory, V.M. Garrett, S.A. Hawkins, K.G. Robinson et G.S. Sayler. 2003. Real-time PCR quantification of nitrifying bacteria in a municipal wastewater treatment plant. Environmental Science & Technology 37:343-351.

Harrison, M.J. 1996. A sugar transporter from *Medicago truncatula* - altered expression pattern in roots during vesicular-arbuscular (VA) mycorrhizal associations. Plant Journal 9:491-503.

Harrison, M.J. et M.L. van Buuren. 1995. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. Nature 378:626-629.

Harrison, M.J. 2005. Signaling in the arbuscular mycorrhizal symbiosis. Annual Review of Microbiology 59:19-42.

Hause, B., W. Maier, O. Miersch, R. Kramell et D. Strack. 2002. Induction of jasmonate biosynthesis in arbuscular mycorrhizal barley roots. Plant Physiology 130:1213-1220.

Hause, B. et T. Fester. 2005. Molecular and cell biology of arbuscular mycorrhizal symbiosis. Planta 221:184-196.

Hause, B., C. Mrosk, S. Isayenkov et D. Strack. 2007. Jasmonates in arbuscular mycorrhizal interactions. Phytochemistry 68:101-110.

Hawes, M.C. 1990. Living plant cells released from the root cap: a regulator of microbial populations in the rhizosphere? Plant & Soil 129:19-27.

Hawkins, H.J., A. Johansen et E. George. 2000. Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. Plant & Soil 226:275-285.

Head, I.M., J.R. Saunders et R.W. Pickup. 1998. Microbial evolution, diversity, and ecology - a decade of ribosomal RNA analysis of uncultivated microorganisms. Microbial Ecology 35:1-21.

Helal, H.M. et D.R. Sauerbeck. 1989. Carbon turnover in the rhizospere. Journal of Plant Nutrition & Soil 149:181-188.

Hengstmann, U., K.J. Chin, P.H. Janssen et W. Liesack. 1999. Comparative phylogenetic assignment of environmental sequences of genes encoding 16S rRNA and numerically abundant culturable bacteria from an anoxic rice paddy soil. Applied & Environmental Microbiology 65:5050-5058.

Herrera-Medina, M.J., H. Gagnon, Y. Piché, J.A. Ocampo, J.M. García-Garrido et H. Vierheilig. 2003. Root colonization by arbuscular mycorrhizal fungi is affected by the salicylic acid content of the plant. Plant Science 164:993-998.

Hewitt, E.J. 1966. Sand and water culture methods used in the study of plant nutrition London, UK: CAB.

Heyrman, J., N.A. Logan, M. Rodríguez-Díaz, P. Scheldeman, L. Lebbe, J. Swings, M. Heyndrickx et P. De Vos. 2005. Study of mural painting isolates, leading to the transfer of '*Bacillus maroccanus*' and '*Bacillus carotarum*' to *Bacillus simplex*, emended description of *Bacillus simplex*, re-examination of the strains previously attributed to '*Bacillus macroides*' and description of *Bacillus muralis* sp. nov. International Journal of Systematic & Evolutionary Microbiology 55:119-131.

Hickman, C.J. 1970. Biology of Phytophthora zoospores. Phytopathology 60:1128-1135.

Hijri, M. et I.R. Sanders. 2005. Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. Nature 433:160-163.

Hildebrandt, U., F. Ouziad, F.J. Marner et H. Bothe. 2006. The bacterium *Paenibacillus validus* stimulates growth of the arbuscular mycorrhizal fungus *Glomus*

intraradices up to the formation of fertile spores. FEMS Microbiology Letters 254:258-267.

Hildebrandt, U., M. Regvar et H. Bothe. 2007. Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68:139-146.

Hill, A.E., D.E. Grayson et J.W. Deacon. 1998. Suppressed germination and early death of *Phytophthora infestans* sporangia caused by pectin, inorganic phosphate, ion chelators and calcium-modulating treatments. European Journal of Plant Pathology 104:367-376.

Hill, G.T., N.A. Mitkowski, L. Aldrich-Wolfe, L.R. Emele, D.D. Jurkonie, A. Ficke, S. Maldonado-Ramirez, S.T. Lynch et E.B. Nelson. 2000. Methods for assessing the composition and diversity of soil microbial communities. Applied Soil Ecology 15:25-36.

Hiltner, L. 1904. Über neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteruiligie und unter besonderer Berücksichtiguang der Gründüngung und Brache. Arb. Deutsch Landwirt. Ges. 98:59-78.

Ho, H.H. et C.J. Hickman. 1967a. Asexual reproduction and behavior of zoospores of *Phytophthora megasperma* var. *sojae*. Canadian Journal of Botany 45:1963-1981.

Ho, H.H. et C.J. Hickman. 1967b. Factors governing zoospore responses of *Phytophthora megasperma* var. *sojae* to plant roots. Canadian Journal of Botany 45:1983-1994.

Hodge, A. 2000. Microbial ecology of the arbuscular mycorrhiza. FEMS Microbiology Ecology 32:91-96.

Hodge, A., S.J. Grayston et B.G. Ord. 1996. A novel method for characterisation and quantification of plant root exudates. Plant & Soil 184:97-104.

Hodge, A., E. Paterson, S.J. Grayston, C.D. Campbell, B.G. Ord et K. Killham. 1998. Characterisation and microbial utilisation of exudate material from the rhizosphere of *Lolium perenne* grown under CO_2 enrichment. Soil Biology & Biochemistry 30:1033-1043.

Hong, T.Y. et M. Meng. 2003. Biochemical characterization and antifungal activity of an endo-1,3-beta-glucanase of *Paenibacillus* sp. isolated from garden soil. Applied Microbiology & Biotechnology 61:472-478.

Hooker, J.E., M. Jaizme-Vega et D. Atkinson. 1994. Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. Dans *Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems* (ed. S. Gianinazzi et H. Schüepp), pp. 191-200. Birkhäuser: Verlag Basel.

Hooker, J.E., P. Piatti, M.V. Cheshire, C.A. Watson. 2007. Polysaccharides and monosaccharides in the hyphosphere of the arbuscular mycorrhizal fungi *Glomus* E3 and *Glomus tenue*. Soil Biology & Biochemistry 39:680-683.

Ibekwe, A.M. et A.C. Kennedy. 1998. Fatty acid methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils. Plant & Soil 206:151-161.

Isayenkov, S., C. Mrosk, I. Stenzel, D. Strack et B. Hause. 2005. Suppression of allene oxide cyclase in hairy roots of *Medicago truncatula* reduces jasmonate levels and the degree of mycorrhization with *Glomus intraradices*. Plant Physiology 139:1401-1410.

Jakobsen, I., L.K. Abbott et A.D. Robson. 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. New Phytologist 12:371-380.

Jakobsen, I., S. Smith et F. Smith. 2002. Function and diversity of arbuscular mycorrhizae in carbon and mineral nutrition. Dans *Mycorrhizal Ecology* (ed. M. G. A. van der Heijden et I. R. Sanders), pp. 75-92. Berlin: Springer-Verlag.

Jargeat, P., C. Cosseau, B. Ola'h, A. Jauneau, P. Bonfante, J. Batut et G. Bécard. 2004. Isolation, free-living capacities, and genome structure of "*Candidatus* glomeribacter gigasporarum," the endocellular bacterium of the mycorrhizal fungus *Gigaspora margarita*. Journal of Bacteriology 186:6876-6884.

Jastrow, J.D., R.M. Miller et J. Lussenhop. 1998. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biology & Biochemistry 30:905-916.

Jeffries, P. et J.C. Dodd. 1996. Functional ecology of mycorrhizal fungi in sustainable soil-plant systems. Dans *Mycorrhizas in integrated systems from genes to plant development* (ed. C. Azcón-Aguilar et J. M. Barea), pp. 497–501. Brussels: European Commission.

Jeffries, P., S. Gianinazzi, S. Perotto, K. Turnau et J.M. Barea. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biology & Fertility of Soils 37: 1-16.

Jentschel, K., D. Thiel, F. Rehn et J. Ludwig-Müller. 2007. Arbuscular mycorrhiza enhances auxin levels and alters auxin biosynthesis in *Tropaeolum majus* during early stages of colonization. Physiologia Plantarum 129:320-333.

Jin, H., P.E. Pfeffer, D.D. Douds, E. Piotrowski, P.J. Lammers et Y. Shachar-Hill. 2005. The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorrhizal symbiosis. New Phytologist 168:687-696.

Johansson, J.F., L.R. Paul et R.D. Finlay. 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiology Ecology 48:1-13.

Johnson, C.R., J.A. Menge, S. Schab et I.P. Ting. 1982. Interaction of photoperiod and vesicular-arbuscular mycorrhizae on growth and metabolism of sweet orange. New Phytologist 90:665-669.

Johnson, D., J.R. Leake et D.J. Read. 2002. Transfer of recent photosynthate into mycorrhizal mycelium of an upland grassland: short-term respiratory losses and accumulation of C-14. Soil Biology & Biochemistry 34:1521-1524.

Jolicoeur, M., E. Bouchard-Marchand, G. Bécard et M. Perrier. 2002. Regulation of mycorrhizal symbiosis: development of a structured nutritional dual model. Ecological Modelling 158:121-142.

Jones, S.W., S.P. Donaldson et J.W. Deacon. 1991. Behaviour of zoospores and zoospore cysts in relation to root infection by *Pythium aphannidermatum*. New Phytologist 117:289-301.

Jones, D.L. et P.R. Darrah. 1993b. Influx and efflux of amino acids from *Zea mays* L. roots and their implications for N nutrition and rhizosphere. Plant & Soil 153:47-59.

Jones, D.L. et P.R. Darrah. 1993c. Re-sorptin of organic compounds by roots of *Zea mays* L. and its consequences in the rhizosphere. Plant & Soil 153:47-59.

Jones, A., A. Prabowo et L. Kochian. 1996. Kinetics of malate transport and decomposition in acid soils and isolated bacterial populations: The effect of

microorganisms on root exudation of malate under Al stress. Plant & Soil 182:239-247.

Jones, D.L. 1998. Organic acids in the rhizosphere - a critical review. Plant & soil 205:25-44.

Jones, D.L., A. Hodge et Y. Kuzyakov. 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytologist 163:459-480.

Jung, W.J., Y.L. Jin, K.Y. Kim, R.D. Park et T.H. Kim. 2005. Changes in pathogenesis-related proteins in pepper plants with regard to biological control of phytophthora blight with *Paenibacillus illinoisensis*. BioControl 50:165-178.

Kabir, Z., I.P. OHalloran, J.W. Fyles et C. Hamel. 1997. Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization: Hyphal density and mycorrhizal root colonization. Plant & Soil 192:285-293.

Kaiser, O., A. Pühler et W. Selbitschka. 2001. Phylogenetic analysis of microbial diversity in the rhizoplane of oilseed rape (*Brassica napus* cv. *Westar*) employing cultivation-dependent and cultivation-independent approaches. Microbial Ecology 42:136-149.

Kaldorf, M. et J. Ludwig-Müller. 2000. AM fungi might affect the root morphology of maize by increasing indole-3-butyric acid biosynthesis. Physiologia Plantarum 109:58-67.

Kapoor, R., M. Kaur et K.G. Mukerji. 2000. VAM and phospohrus induced changes in the rhizosphere ecology of *Anethum graveolens* L. Journal of Experimental Biology 21:185-191.

Karagiannidis, N., F. Bletsos et N. Stavropoulos. 2002. Effect of Verticillium wilt (*Verticillium dahliae* Kleb.) and mycorrhiza (*Glomus mosseae*) on root colonization, growth and nutrient uptake in tomato and eggplant seedlings. Scientia Horticulturae 94:145-156.

Karandashov, V. et M. Bucher. 2005. Symbiotic phosphate transport in arbuscular mycorrhizas. Trends in Plant Science 10:22-29.

Khew, K.L. et G.A. Zentmyer. 1973. Chemotactic response of zoospores of five species of Phytophthora. Phytopathology 63:1511-1517.

Kishor, P.B.K., S. Sangam, R.N. Amrutha, P.S. Laxmi, K.R. Naidu, K. Rao, S. Rao, K.J. Reddy, P. Theriappan et N. Sreenivaslu. 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Current Science 88:424-438.

Klironomos, J.N., M. Ursic, M. Rillig et M.F. Allens. 1998. Interspecific differences in the response of arbuscular mycorrhizal fungi to *Artemisia tridentata* grown under elevated atmospheric CO2. New Phytologist 138:599-605.

Klironomos, J.N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67-70.

Knee, E.M., F.C. Gong, M.S. Gao, M. Teplitski, A.R. Jones, A. Foxworthy, A.J. Mort et W.D. Bauer. 2001. Root mucilage from pea and its utilization by rhizosphere bacteria as a sole carbon source. Molecular Plant-Microbe Interactions 14:775-784.

Koide, R.T. et Z. Kabir. 2000. Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. New Phytologist 148:511-517.

Kormanik, P.P. et A.C. Mc Graw. 1982. Quantification of vesicular-arbuscular mycorrhizae. Dans *Methods and principles of mycorrhizal research*, (ed. Schenck, N. C.), pp 37-45. St-Paul, Minnesota: The American Phytopathological Society Press.

Kozdrój, J. et J.D. van Elsas. 2000. Response of the bacterial community to root exudates in soil polluted with heavy metals assessed by molecular and cultural approaches. Soil Biology & Biochemistry 32:1405-1417.

Krishna, K.R. et D.J. Bagyaraj. 1983. Interaction between *Glomus fasciculatum* and *Sclerotium rolfsi*i in peanut. Canadian Journal of Botany 61:2349-2351.

Kuznetsov, V.V. et N.I. Shevyakova. 1999. Proline under stress: biological role, metabolism, and regulation. Russian Journal of Plant Physiology 46:274-287.

Kuzyakov, Y. et G. Domanski. 2000. Carbon input by plants into the soil. Journal of Plant Nutrition & Soil Science 163:421-431.

Labour, K., M. Jolicoeur et M. St-Arnaud. 2003. Arbuscular mycorrhizal responsiveness of *in vitro* tomato root lines is not related to growth and nutrient uptake rates. Canadian Journal of Botany 81:645-656.

Lammers, P.J., J. Jun, J. Abubaker, R. Arreola, A. Gopalan, B. Bago, C. Hernandez-Sebastia, J.W. Allen, D.D. Douds, P.E. Pfeffer et Y. Shachar-Hill. 2001. The glyoxylate cycle in an arbuscular mycorrhizal fungus. Carbon flux and gene expression. Plant Physiology 127:1287-1298.

Larkin, R.P. et D.R. Fravel. 1999. Mechanisms of action and dose-response relationships governing biological control of Fusarium wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 89:1152-1161.

Larsen, J. et I. Jakobsen. 1996. Interactions between a mycophagous *Collembola*, dry yeast and the external mycelium of an arbuscular mycorrhizal fungus. Mycorrhiza 6:259-264.

Larsen, J. et L. Bødker. 2001. Interactions between pea root-inhabiting fungi examined using signature fatty acids. New Phytologist 149:487-493.

Larsen, J., P.A. Olsson et I. Jakobsen. 1998. The use of fatty acid signatures to study mycelial interactions between the arbuscular mycorrhizal fungus *Glomus intraradices* and the saprotrophic fungus *Fusarium culmorum* in root-free soil. Mycological Research 102:1491-1496.

Larsen, J., S. Ravnskov et I. Jakobsen. 2003. Combined effect of an arbuscular mycorrhizal fungus and a biocontrol bacterium against *Pythium ultimum* in soil. Folia Geobotanica 38:145-154.

Leaño, E.M., L.L.P. Vrijmoed et E.B.G. Jones. 1998. Zoospore chemotaxis of two mangrove strains of *Halophytophthora vesicula* from Mai Po, Hong Kong. Mycologia 90:1001-1008.

Lebuhn, M., T. Heulin et A. Hartmann. 1997. Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from different proximity to plant roots. FEMS Microbiology Ecology 22:325-334.

Lee, K.E. et C.E. Parkhurst. 1992. Soil organisms and sustainable productivity. Australian Journal of Soil Ressources 30:855-892.

Lee, D.H., Y.G. Zo et S.J. Kim. 1996. Non-radioactive method to study genetic profiles of natural bacterial communities by PCR-single-strand-conformation polymorphism. Applied & Environmental Microbiology 62:3112-3120.

Levy, Y. et J. Krikun. 1980. Effect of vesicular-arbuscular mycorrhiza on *Citrus jambhiri* water relations. New Phytologist 85:25-31.

Levy, A., B.J. Chang, L.K. Abbott, J. Kuo, G. Harnett et T.J.J. Inglis. 2003. Invasion of spores of the arbuscular mycorrhizal fungus *Gigaspora decipiens* by *Burkholderia* spp. Applied & Environmental Microbiology 69:6250-6256.

Leyval, C., K. Turnau et K. Haselwandter. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function - physiological, ecological and applied aspects. Mycorrhiza 7:139-153.

Lidström, M.E. et L. Chistoserdova. 2002. Plants in the pink: cytokinin production by *Methylobacterium*. Journal of Bacteriology 184:1818.

Liljeroth, E., P.J. Kuikman et L.A. Van Veen. 1994. Carbon translocation to the rhizosphere of maize and wheat and influence on the turnover of native soil organic matter at different soil nitrogen levels. Plant & Soil 161:233-240.

Linderman, R.G. 1988. Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. Phytopathology 78:366-371.

Linderman, R.G. 1992. Vesicular-arbuscular mycorrhizae and soil microbial interactions. Dans *Mycorrhizae in sustainable agriculture*, Special Publ. No. 54 (ed. G. J. Bethlenfalvay et R. G. Linderman), pp. 45-70. Madison, Wisconsin: The American Society of Agronomy.

Linderman, R.G. 1994. Role of VAM fungi in biocontrol. Dans *Mycorrhizae and plant health* (ed. F. L. Pfleger et R. G. Linderman), pp. 1-25. St. Paul, Minnesota: The Amercian Phytopathological Press.

Linderman, R.G. 2000. Effects of mycorrhizas on plant tolerance to diseases. Dans *Arbuscular mycorrhizas: physiology and functions* (ed. Y. Kapulnik et D. Douds), pp. 345-365. The Netherlands: Kluwer Academic Publishers.

Linderman, R.G. 2001. Mycorrhizae and their effects on diseases. Dans *Diseases of woody ornamentals and trees in nurseries* (ed. R. K. Jones et D. M. Benson), pp. 433-434. St Paul, Minnesota: APS Press.

Linderman, R.G. et T.C. Paulitz. 1990. Mycorrhizal-rhizobacterial interactions. Dans *Biological control of soil-borne plant pathogens* (ed. D. Hornby, R. J. Cook, Y.

Henis, W. H. Ko, A. D. Rovira, B. Schippers et P. R. Scott), pp. 261-283. Wallingford, UK: CAB International.

Lingua, G., A. Fusconi et G. Berta. 2001. The nucleus of differentiated root plant cells: modifications induced by arbuscular mycorrhizal fungi. European Journal of Histochemistry 45:9-20.

Liu, A., C. Hamel, R.I. Hamilton, B.L. Ma et D.L. Smith. 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. Mycorrhiza 9:331-336.

Ludwig-Müller, J. 2000a. Hormonal blance in plants during colonization by mycorrhizal fungi. Dans *Arbuscular Mycorrhizas: Physiology and function* (ed. Y. Kapulnik, et D. D. Douds), pp. 263-285. Dordrecht, The Netherlands: Kluwer Academic Publisher.

Ludwig-Müller, J. 2000b. Indole-3-butyric acid in plant growth and development. Plant Growth Regulation 32:219-230.

Ludwig-Müller, J., R.N. Bennett, J.M. García-Garrido, Y. Piché et H. Vierheilig. 2002. Reduced arbuscular mycorrhizal root colonization in *Tropaeolum majus* and *Carica papaya* after jasmonic acid application can not be attributed to increased glucosinolate levels. Journal of Plant Physiology 159:517-523.

Lugtenberg, B.J.J., L.V. Kravchenko et M. Simons. 1999. Tomato seed and root exudate sugars: composition, utilization by *Pseudomonas* biocontrol strains and role in rhizosphere colonization. Environmental Microbiology 1:439-446.

Lugtenberg, B.J.J., L. Dekkers et G.V. Bloemberg. 2001. Molecular determinants of rhizosphere colonization by *Pseudomonas*. Annual Review of Phytopathology 39:461-490.

Lugtenberg, B. J. J., A. Chin, T. F. C. Woeng, G. V. Bloemberg. 2002. Microbe-plant interactions: principles and mechanisms. Journal of General & Molecular Microbiology 81:373-383.

Lynch, J.M. et J.M. Whipps. 1990. Substrate flow in the rhizosphere. Plant & Soil 129:1-10.

MacDonald, R.M., M.R. Chandler et B. Mosse. 1982. The occurrence of bacterium-like organelles in vesicular-arbuscular mycorrhizal fungi. New Phytologist 90:659-663.

Maldonado-Mendoza, I.E., G.R. Dewbre et M.J. Harrison. 2001. A phosphate transporter gene from the extra-radical mycelium of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment. Molecular Plant-Microbe Interactions 14:1140-1148.

Mandal, N.C. et A.K. Sinha. 1991. Differential effects of root exudates and extracts from tomato cultivars on *Fusarium oxysporum* f. sp. *lycopersic*i in relation to their disease reaction. Journal of Mycopathological Research 29:9-16.

Mandelbaum, C.I. et Y. Piché. 2000. The role of root exudates in arbuscular mycorrhiza initiation. Dans *Mycorrhizal Biology,* (ed. Mukerji, K.G., Chamola, B.P., Singh, J.), pp. 153-172. New York: Kluwer Academic Press.

Manjaiah, K.M., R.P. Voroney et U. Sen. 2000. Soil organic carbon stocks, storage profile and microbial biomass under different crop management systems in a tropical agricultural ecosystem. Biology & Fertility of Soils 32:273-278.

Mansfeld-Giese, K., J. Larsen et L. Bødker. 2002. Bacterial populations associated with mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*. FEMS Microbiology Ecology 41:133-140.

Marschener, H. 1998. Role of root growth, arbuscular mycorrhiza, and root exudates for the efficiency in nutrient acquisition. Field Crops Research 56:203-207.

Marschner, H. 1986. Mineral nutrition of higher plants. London: Academic Press.

Marschner, H. 1996. Mineral nutrient acquisition in nonmycorrhizal and mycorrhizal plants. Phyton Annales Rei Botanicae 36:61-68.

Marschner, P. et D.E. Crowley. 1996a. Physiological activity of a bioluminescent *Pseudomonas fluorescens* (strain 2-79) in the rhizosphere of mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum* L). Soil Biology & Biochemistry 28:869-876.

Marschner, P. et D.E. Crowley. 1996b. Root colonization of mycorrhizal and nonmycorrhizal pepper (*Capsicum annuum*) by *Pseudomonas fluorescens* 2-79RL. New Phytologist 134:115-122.

Marschner, P. et K. Baumann. 2003. Changes in bacterial community structure induced by mycorrhizal colonisation in split-root maize. Plant & Soil 251:279-289.

Marschner, P. et S. Timonen. 2005. Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. Applied Soil Ecology 28:23-36.

Marschner, P., D.E. Crowley et R.M. Higashi. 1997. Root exudation and physiological status of a root-colonizing fluorescent pseudomonad in mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum* L). Plant & Soil 189:11-20.

Marschner, P., D.E. Crowley et R. Lieberei. 2001. Arbuscular mycorrhizal infection changes the bacterial 16 S rDNA community composition in the rhizosphere of maize. Mycorrhiza 11:297-302.

Martinez, A., M. Obertello, A. Pardo, J.A. Ocampo et A. Godeas. 2004. Interactions between *Trichoderma pseudokoningii* strains and the arbuscular mycorrhizal fungi *Glomus mosseae* and *Gigaspora rosea*. Mycorrhiza 14:79-84.

Mavingui, P. et T. Heulin. 1994. *In vitro* chitinase and antifungal activity of a soil, rhizosphere and rhizoplane population of *Bacillus polymyxa*. Soil Biology & Biochemistry 26:801-803.

Mayo, K., R.E. Davis et J. Motta. 1986. Stimulation of germination of spores of *Glomus versiforme* by spore-associated bacteria. Mycologia 78:426-431.

Mazzola, M. 2002. Mechanisms of natural soil suppresiveness to soilborne diseases. Journal of General & Molecular Microbiology 81:557-564.

McAllister, C.B., I. García-Romera, A. Godeas et J.A. Ocampo. 1994. Interactions between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae*: effects on plant growth, arbuscular mycorrhizas and the saprophyte inoculants. Soil Biology & Biochemistry 26:1363-1367.

McAllister, C.B., I. García-Romera, J. Martín, A. Godeas et J.A. Ocampo. 1995. Interaction between *Aspergillus niger* van Tiegh and *Glomus mosseae* (Nicol and Gerd) Gerd and Trappe. New Phytologist 129:309-316.

McDougall, B.M. 1970. Movement of 14C photosynthetate into the roots of wheat seedings and exudation of 14C from intact roots. New Phytologist 69:999-1003.

Medina, A., N. Vassilev, M.M. Alguacil, A. Roldan et R. Azcón. 2004a. Increased plant growth, nutrient uptake, and soil enzymatic activities in a desertified mediterranean soil amended with treated residues and inoculated with native mycorrhizal fungi and a plant growth-promoting yeast. Soil Science 169:260-270.

Medina, A., M. Vassileva, F. Caravaca, A. Roldan et R. Azcón. 2004b. Improvement of soil characteristics and growth of *Dorycnium pentaphyllum* by amendment with agrowastes and inoculation with AM fungi and/or the yeast *Yarowia lipolytica*. Chemosphere 56:449-456.

Meharg, A.A. et K. Killham. 1995. Loss of exudates from the roots of perennial ryegrass inoculated with a range of micro-organisms. Plant & Soil 170:345-349.

Meixner, C., J. Ludwig-Müller, O. Miersch, P. Gresshoff, C. Staehelin et H. Vierheilig. 2005. Lack of mycorrhizal autoregulation and phytohormonal changes in the supernodulating soybean mutant nts1007. Planta 222:709-715.

Meyer, J.R. et R.G. Linderman. 1986a. Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomyces by mycorrhizas formed by *Glomus fasciculatum*. Soil Biology & Biochemistry 18:191-196.

Meyer, J.R. et R.G. Linderman. 1986b. Response of subterranean clover to dual inoculation with vesicular-arbuscular mycorrhizal fungi and a plant growth-promoting bacterium, *Pseudomonas putida*. Soil Biology & Biochemistry 18:185-190.

Miethling, R., G. Wieland, H. Backhaus et C.C. Tebbe. 2000. Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L33. Microbial Ecology 40:43-56.

Minerdi, D., R. Fani, R. Gallo, A. Boarino et P. Bonfante. 2001. Nitrogen fixation genes in an endosymbiotic *Burkholderia* strain. Applied & Environmental Microbiology 67:725-732.

Morris, P.F. et E.W.B. Ward. 1992. Chemoattraction of zoospores of the soybean pathogen, *Phytophthora sojae*, by isoflavones. Physiological & Molecular Plant Pathology 40:17-22.

Morris, B.M., B. Reid et N.A.R. Gow. 1995. Tactic response of zoospores of the fungus *Phytophthora palmivora* to solutions of different pH in relation to plant infection. Microbiology 141:1231-1237.

Muyzer, G., E.C. de Waal et A.G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied & Environmental Microbiology 59:695-700.

Muyzer, G. 1999. DGGE/TGGE a method for identifying genes from natural ecosystems. Current Opinion in Microbiology 2:317-322.

Muyzer, G. et K. Smalla. 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. International Journal of General & Molecular Microbiology 73:127-141.

Nakano, A., K. Takahashi et M. Kimura. 1999. The carbon origin of arbuscular mycorrhizal fungi estimated from delta C-13 values of individual spores. Mycorrhiza 9:41-47.

Nehl, D.B., S.J. Allen et J.F. Brown. 1997. Deleterious rhizosphere bacteria: an integrating perspective. Applied Soil Ecology 5:1-20.

Nelsen, C.E. et G.R. Safir. 1982. Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta 154:407-413.

Nemec, S. 1994. Soil microflora associated with pot cultures of *Glomus intraradices*-infected *Citrus reticulata*. Applied Soil Ecology 1:299-306.

Nguyen, C. 2003. Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23:375-396.

Nielsen, P. et J. Sørensen. 1997. Multi-target and medium-independent fungal antagonism by hydrolytic enzymes in *Paenibacillus polymyxa* and *Bacillus pumilus* strains from barley rhizosphere. FEMS Microbiology Ecology 22:183-192.

Norman , J.R., D. Atkinson et Hooker. 1996. Arbuscular mycorrhizal fungi-induced alteration to root architecture in strawberry and induced resistance to the root pathogen *Phytophthora fragariae*. Plant & Soil 185: 191-198.

Norman, J.R. et J.E. Hooker. 2000. Sporulation of *Phytophthora fragariae* shows greater stimulation by exudates of non-mycorrhizal than by mycorrhizal strawberry roots. Mycological Research 104:1069-1073.

Oehl, F., E. Sieverding, K. Ineichen, E.A. Ris, T. Boller et A. Wiemken. 2005. Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. New Phytologist 165:273-283.

Ohtomo, R. et M. Saito. 2005. Polyphosphate dynamics in mycorrhizal roots during colonization of an arbuscular mycorrhizal fungus. New Phytologist 167:571-578.

Olsson, P.A., M. Chalet, E. Bååth, R.D. Finlay et B. Söderström. 1996a. Ectomycorrhizal mycelia reduce bacterial activity in a sandy soil. FEMS Microbiology Ecology 21:77-86.

Olsson, P.A., E. Bååth, I. Jakobsen et B. Söderström. 1996b. Soil bacteria respond to presence of roots but not to mycelium of arbuscular mycorrhizal fungi. Soil Biology & Biochemistry 28:463-470.

Olsson, P.A., R. Francis, D.J. Read et B. Söderström. 1998. Growth of arbuscular mycorrhizal mycelium in calcareous dune sand and its interaction with other soil microorganisms as estimated by measurement of specific fatty acids. Plant & Soil 201:9-16.

Olsson, P.A., I. Thingstrup, I. Jakobsen et F. Bååth. 1999. Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field. Soil Biology & Biochemistry 31:1879-1887.

Omokolo, N.D., D.J. Nankeu, N. Niemenak et P.F. Djocgoue. 2002. Analysis of amino acids and carbohydrates in the cortex of nine clones of *Theobroma cacao* L. in relation to their susceptibility to *Phytophthora megakarya* Bra. and Grif. Crop Protection 21:395-402.

Park, S., Y. Takano, H. Matsuura et T. Yoshihara. 2004. Antifungal compounds from the root and root exudate of *Zea mays*. Bioscience, Biotechnology & Biochemistry 68:1366-1368.

Paszkowski, U. 2006. A journey through signaling in arbuscular mycorrhizal symbioses 2006. New Phytologist 172:35-46.

Paul, E.A. et F.E. Clark. 1996. Soil microbiology and biochemistry Academic Press, San Diego.

Paulitz, T.C. et R.G. Linderman. 1989. Interactions between fluorescent pseudomonads and VA mycorrhizal fungi. New Phytologist 113:37-45.

Pearson, J.N. et I. Jakobson. 1993. The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants, measured by dual labelling with ^{32}P and ^{33}P. New Phytologist 124:489-494.

Pennanen, T., S. Caul, T.J. Daniell, B.S. Griffiths, K. Ritz et R.E. Wheatley. 2004. Community-level responses of metabolically-active soil microorganisms to the quantity and quality of substrate inputs. Soil Biology & Biochemistry 36:841-848.

Perrin, R. 1990. Interactions between mycorrhizae and diseases caused by soil-borne fungi. Soil Use Management 6: 189-195.

Peters, N.K. et S.R. Long. 1988. Alfalfa root exudates and compounds which promote or inhibit induction of *Rhizobium meliloti* nodulation genes. Plant Physiology 88:396-400.

Peters, N.K. et D.P.S. Verma. 1990. Phenolic compounds as regulators of gene expression in plant-microbe interactions. Molecular Plant-Microbe Interactions 3:4-8.

Pettersson, B., K.E. Rippere, A.A. Yousten et F.G. Priest. 1999. Transfer of *Bacillus lentimorbus* and *Bacillus popilliae* to the genus *Paenibacillus* with emended descriptions of *Paenibacillus lentimorbus* comb. nov., and *Paenibacillus popilliae* comb. nov. International Journal of Systematic Bacteriology 49:531-540.

Pfeffer, P.E., D.D. Douds, G. Bécard et Y. Shachar-Hill. 1999. Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza. Plant Physiology 120:587-598.

Piga, P.M., R.R. Bélanger, T.C. Paulitz et N. Benhamou. 1997. Increased resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63-28. Physiological & Molecular Plant Pathology 50:301-320.

Pinior, A., U. Wyss, Y. Piché et H. Vierheilig. 1999. Plants colonized by AM fungi regulate further root colonization by AM fungi through altered root exudation. Canadian Journal of Botany 77:891-897.

Pinior, A., G. Grunewaldt-Stöcker, H. von Alten et R.J. Strasser. 2005. Mycorrhizal impact on drought stress tolerance of rose plants probed by chlorophyll a fluorescence, proline content and visual scoring. Mycorrhiza 15:596-605.

Pinton, R., Z. Varanini et P. Nannipieri. 2001. The Rhizosphere. Biochemistry and organic substances at the soil-plant interface. New York: Marcel Dekker, Inc.

Piuri, M., C. Sanchezrivas et S.M. Ruzal. 1998. A novel antimicrobial activity of a *Paenibacillus polymyxa* strain isolated from regional fermented sausages. Letters in Applied Microbiology 27:9-13.

Posta, K., H. Marschner et V. Romheld. 1994. Manganese reduction in the rhizosphere of mycorrhizal and nonmycorrhizal maize. Mycorrhiza 5:119-124.

Poulin, M.J., R. Bel-Rhlid, Y. Piché et R. Chenevert. 1993. Flavonoids released by carrot (*Daucus carota*) seedlings stimulate hyphal development of vesicular-arbuscular mycorrhiza; fungi in the presence of optimal CO_2 enrichment. Journal of Chemical Ecology 19:2317-2326.

Pozo, M.J., E. Dumas-Gaudot, S. Slezack, C. Cordier, A. Asselin, S. Gianinazzi, V. Gianinazzi-Pearson, C. Azcón-Aguilar et J.M. Barea. 1996. Induction of new chitinase isoforms in tomato roots during interactions with *Glomus mosseae* and/or *Phytophthora nicotianae* var *parasitica*. Agronomie 16:689-697.

Pozo, M.J., C. Azcón-Aguilar, E. Dumas-Gaudot et J.M. Barea. 1998. Chitosanase and chitinase activities in tomato roots during interactions with arbuscular mycorrhizal fungi or *Phytophthora parasitica*. Journal of Experimental Botany 49:1729-1739.

Pozo, M.J., C. Azcón-Aguilar, E. Dumas-Gaudot et J.M. Barea. 1999. ß-1,3-glucanase activities in tomato roots inoculated with arbuscular mycorrhizal fungi and/or *Phytophthora parasitica* and their possible involvement in bioprotection. Plant Science 141:149-157.

Pozo, M.J., C. Cordier, E. Dumas-Gaudot, S. Gianinazzi, J.M. Barea et C. Azcón-Aguilar. 2002a. Localized versus systemic effect of arbuscular mycorrhizal fungi

on defence responses to Phytophthora infection in tomato plants. Journal of Experimental Botany 53:525-534.

Pozo, M.J., S. Slezack-Deschaumes, E. Dumas-Gaudot, S. Gianinazzi et C. Azcón-Aguilar. 2002b. Plant defense responses induced by arbuscular mycorrhizal fungi. Dans *Mycorrhizal technology in agriculture: from genes to bioproducts* (ed. S. Gianinazzi H. Schuepp J. M. Barea et K. Haselwandter), pp. 103-111. Basel, Switzerland: Birkhauser Verlag Ag.

Pozo, M.J., L.C. Van Loon et C.M.J. Pieterse. 2004. Jasmonates - Signals in plant-microbe interactions. Journal of Plant Growth Regulation 23:211-222.

Puppi, G., R. Azcón et G. Hoflich. 1994. Management of positive interactions of arbuscular mycorrhizal fungi with essential groups of soil microorganisms. In *Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems* (ed. S. Gianinazzi et H. Schuepp), pp. 201-216. Basel, Switzerland: Birkhauser Verlag.

Rai, V.K. 2002. Role of amino acids in plant responses to stresses. Biologia Plantarum 45:481-487.

Raiesi, F. et M. Ghollarata. 2006. Interactions between phosphorus availability and an AM fungus (*Glomus intraradices*) and their effects on soil microbial respiration, biomass and enzyme activities in a calcareous soil. Pedobiologia 50:413-425.

Ratnayake, M., R.T. Leonard et J. Menge. 1978. Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. New Phytologist 81:543-552.

Ravnskov, S., O. Nybroe et I. Jakobsen. 1999. Influence of an arbuscular mycorrhizal fungus on *Pseudomonas fluorescens* DF57 in rhizosphere and hyphosphere soil. New Phytologist 142:113-122.

Ravnskov, S., J. Larsen et I. Jakobsen. 2002. Phosphorus uptake of an arbuscular mycorrhizal fungus is not effected by the biocontrol bacterium *Burkholderia cepacia*. Soil Biology & Biochemistry 34:1875-1881.

Read, D.J. 1991. Mycorrhizae in ecosystems. Experimentia 47:376-391.

Redecker, D., J.B. Morton et T.D. Bruns. 2000. Ancestral lineages of arbuscular mycorrhizal fungi (Glomales). Molecular Phylogenetics & Evolution 14:276-284.

Reid, C.P.P. et J.G. Mexal. 1977. Water stress effects on root exudation by lodgepole pine. Soil Biology & Biochemistry 9:417-421.

Reid, B., B.M. Morris et N.A.R. Gow. 1995. Calcium-dependent, genus-specific, autoaggregation of zoospores of phytopathogenic fungi. Experimental Mycology 19:202-213.

Remy, W., T.N. Taylor, H. Hass et H. Kerp. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proceedings of the National Academy of Science, U.S.A. 91:11841-11843.

Requena, N., I. Jimenez, M. Toro et J.M. Barea. 1997. Interactions between plant-growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and *Rhizobium* spp. in the rhizosphere of *Anthyllis cytisoides*, a model legume for revegetation in mediterranean semi-arid ecosystems. New Phytologist 136:667-677.

Requena, N., E. Serrano, A. Ocón et M. Breuninger. 2007. Plant signals and fungal perception during arbuscular mycorrhiza establishment. Phytochemistry 68:33-40.

Rhodes, L.H. et J.W. Gerdemann. 1975. Phosphate utake zones of mycorrhizal and non-mycorrhizal onions. New Phytologist 75:555-561.

Ribeiro, O.K. 1983. Physiology of asexual sporulation and spore germination in *Phytophthora*. Dans *Phytophthora: its biology, taxonomy, ecology and pathology*, (ed. Erwin, D. C., S. Bartnicki-Garcia, et P. H. Tsao), St-Paul, Minnesota: The American Phytopathological Society Press.

Richard, C. et G. Boivin. 1994. Maladies et ravageurs des cultures légumières au Canada.Ottawa, Ontario: The Canadian Phytopathological Society.

Richardson, A.E. 1994. Soil microorganisms and phosphorus availability. Dans *Soil biota management in sustainable farming systems* (ed. C. Pankhurst, B. Doube, V. Gupta and P. Grace), pp. 50-62. Melbourne: CSIRO Publishing.

Richardson, A.E. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Australian Journal of Plant Physiology 28:897–906.

Rillig, M.C., E.R. Lutgen, P.W. Ramsey, J.N. Klironomos et J.E. Gannon. 2005. Microbiota accompanying different arbuscular mycorrhizal fungal isolates influence soil aggregation. Pedobiologia 49:251-259.

Rivas, R., C. Gutiérrez, A. Abril, P.F. Mateos, E. Martínez-Molina, A. Ventosa et E. Velázquez. 2005. *Paenibacillus rhizosphaerae* sp. nov., isolated from the rhizosphere of *Cicer arietinum*. International Journal of Systematic & Evolutionary Microbiology 55:1305-1309.

Rivera, R. et K. Fernáncez. 2003. Bases Cientifico. Técnicas para el manejo de los sistemas agrícolas micorrízados eficientemente Estudio de caso: El Caribe Instituto Nacional de Ciencias Agricolas, Cuba.

Roesti, D., K. Ineichen, O. Braissant, D. Redecker, A. Wiemken et M. Aragno. 2005. Bacteria associated with spores of the arbuscular mycorrhizal fungi *Glomus geosporum* and *Glomus constrictum*. Applied & Environmental Microbiology 71:6673-6679.

Rosado, A.S. et L. Seldin. 1993. Production of a potentially novel anti-microbial substance by *Bacillus polymyxa*. World Journal of Microbiology & Biotechnology 9:521-528.

Rousseau, A., N. Benhamou, I. Chet et Y. Piché. 1996. Mycoparasitism of the extramatrical phase of *Glomus intraradices* by *Trichoderma harzianum*. Phytopathology 86: 434-443.

Rovira, A. 1969. Plant root exudates. The Botanical Review 35:55-56.

Royle, D.J. et C.J. Hickman. 1964a. Analysis of factors governing *in vitro* accumulation of zoospores of *Pythium aphanidermatum* on root. I. Behavior of zoospores. Canadian Journal of Botany 10:151-162.

Royle, D.J. et C.J. Hickman. 1964b. Analysis of factors governing *in vitro* accumulation of zoospores of *Pythium aphanidermatum* on roots. II. Substances causing responses. Canadian Journal of Botany 10:201-219.

Ruiz-Lozano, J.M., R. Azcón et M. Gómez. 1995. Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: physiological and nutritional plant responses. Applied & Environmental Microbiology 61:456-460.

Ruiz-Lozano, J.M., R. Azcón et M. Gómez. 1996. Alleviation of salt stress by arbuscular-mycorrhizal *Glomus* species in *Lactuca sativa* plants. Physiologia Plantarum 98:767-772.

Ryan, P.R., E. Delhaize et D.L. Jones. 2001. Function and mechanism of organic anion exudation from plant roots. Annual Review of Plant Physiology & Plant Molecular Biology 52:527-560.

Ryu, C.M., C.H. Hu, R.D. Locy et J.W. Kloepper. 2005. Study of mechanisms for plant growth promotion elicited by rhizobacteria in *Arabidopsis thaliana*. Plant & Soil 268:285-292.

Saito, K., Y. Suyama, S. Sato et K. Sugawara. 2004. Defoliation effects on the community structure of arbuscular mycorrhizal fungi based on 18S rDNA sequences. Mycorrhiza 14:363-373.

Sampedro, I., E. Aranda, J.M. Scervino, S. Fracchia, I. García-Romera, J.A. Ocampo et A. Godeas. 2004. Improvement by soil yeasts of arbuscular mycorrhizal symbiosis of soybean (*Glycine max*) colonized by *Glomus mosseae*. Mycorrhiza 14:229-234.

Sanders, I.R. 2002. Specificity in the arbuscular mycorrhizal symbiosis. Dans *Mycorrhizal ecology* (ed. van der Hejden, M. G. A. et I. R. Sanders), pp 415-437. Berlin: Springer.

Sarwar, M. et R.J. Kremer. 1995. Enhanced suppression of plant growth through production of L-tryptophan-derived compounds by deleterious rhizobacteria. Plant & Soil 172:261-269.

Satour, M.M. et E.E. Butler. 1967. A root and crown rot of tomato caused by *Phytophthora capsici* and *P. parasitica*. Phytopathology 57:510-515.

Scervino, J.M., M.A. Ponce, R. Erra-Bassells, H. Vierheilig, J.A. Ocampo et A. Godeas. 2005a. Arbuscular mycorrhizal colonization of tomato by *Gigaspora* and *Glomus* species in the presence of root flavonoids. Journal of Plant Physiology 162:625-633.

Scervino, J., M. Ponce, R. Erra-Bassells, H. Vierheilig, J. Ocampo et A. Godeas. 2005b. Flavonoids exhibit fungal species and genus specific effects on the presymbiotic growth of *Gigaspora* and *Glomus*. Mycological Research 109:789-794.

Scheffknecht, S., R. Mammerler, S. Steinkellner et H. Vierheilig. 2006. Root exudates of mycorrhizal tomato plants exhibit a different effect on microconidia germination of *Fusarium oxysporum* f. sp. *lycopersici* than root exudates from non-mycorrhizal tomato plants. Mycorrhiza 16:365-370.

Scheffknecht, S., M. St-Arnaud, T. Khaosaad, S. Steinkellner et H. Vierheilig. 2007. An altered root exudation pattern through mycorrhization affecting microconidia germination of the highly specialized tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) is not tomato specific but also occurs in Fol non-host plants. Canadian Journal of Botany. 85: 347-351.

Schellenbaum, L., J. Müller, T. Boller, A. Wiemken et H. Schüepp. 1998. Effects of drought on non-mycorrhizal and mycorrhizal maize - changes in the pools of non-structural carbohydrates, in the activities of invertase and trehalase, and in the pools of amino acids and imino acids. New Phytologist 138:59-66.

Scheublin, T.R., K.P. Ridgway, J.P.W. Young et M.G.A. van der Heijden. 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Applied & Environmental Microbiology 70:6240-6246.

Schilling, G., A. Gransee, A. Deubel, G. Lezovic et S. Ruppel. 1998. Phosphorus availability, root exudates, and microbial activity in the rhizosphere. Journal of Plant Nutrition & Soil Science 161:465-478.

Schüßler, A. 2002. Molecular phylogeny, taxonomy, and evolution of *Geosiphon pyriformis* and arbuscular mycorrhizal fungi. Plant & Soil 244:75-83.

Schüßler, A., D. Schwarzott et C. Walker. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycological Research 105:1413-1421.

Schwab, S.M., J.A. Menge et R.T. Leonard. 1983. Quantitative and qualitative effects of phosphorus on extracts and exudates of sudangrass roots in relation to vesicular-arbuscular mycorrhiza formation. Plant Physiology 73:761-765.

Secilia, J. et D.J. Bagyaraj. 1987. Bacteria and actinomycetes associated with pot cultures of vesicular-arbuscular mycorrhizas. Canadian Journal of Microbiology 33:1069-1073.

Selim, S., J. Negrel, C. Govaerts, S. Gianinazzi et D. van Tuinen. 2005. Isolation and partial characterization of antagonistic peptides produced by *Paenibacillus* sp.

strain B2 isolated from the sorghum mycorrhizosphere. Applied & Environmental Microbiology 71:6501-6507.

Semenov, A., A. van Bruggen et V. Zelenev. 1999. Moving waves of bacterial populations and total organic carbon along roots of wheat. Microbial Ecology 37:116-128.

Sharma, A.K. et B.N. Johri. 2002. Arbuscular-mycorrhiza and plant disease Dans *Arbuscular mycorrhizae: interactions in plants, rhizosphere and soils* (ed. A. K. Shaxena, et B. N. Johri), pp. 69-96. Enfield, New Hampshire: Science Publishers Inc.

Sharma, S.S. et K.J. Dietz. 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. Journal of Experimental Botany 57:711-726.

Shepherd, T. et H.V. Davies. 1994. Patterns of short-term amino acid accumulation and loss in the root-zone of liquid-cultured forage rape (*Brassica napus* L.). Plant & Soil 158:99-109.

Shishido, M., C. Breuil et C.P. Chanway. 1999. Endophytic colonization of spruce by plant growth-promoting rhizobacteria. FEMS Microbiology Ecology 29:191-196.

Siddiqui, Z.A. et I. Mahmood. 1998. Effect of a plant growth promoting bacterium, an AM fungus and soil types on the morphometrics and reproduction of *Meloidogyne javanica* on tomato. Applied Soil Ecology 8:77-84.

Siddiqui, M.A. et S.S. Shaukat. 2002. Mixtures of plant disease suppressive bacteria enhance biological control of multiple tomato pathogens. Biology & Fertility of Soils 36:260-268.

Sievers, A. et M. Braun. 1996. The root cap: structure and function. Dans *Plant roots: the hidden half* (ed. Y. Waisel, A. Eshel et U. Kafkafi), pp. 53-74. New York: Marcel Dekker Inc.

Simon, L., J. Bousquet, R.C. Lévesque et M. Lalonde. 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363:67-69.

Simons, M., H.P. Permentier, L.A. de Weger, C.A. Wijffelman et B.J.J. Lugtenberg. 1997. Amino acids synthesis is necessary for tomato root colonization by

Pseudomonas fluorescens strain WCS365. Molecular Plant Microbe Interactions 10:102-106.

Six, J., H. Bossuyt, S. Degryze et K. Denef. 2004. A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. Soils & Tillage Research 79: 7-31.

Slezack, S., E. Dumas-Gaudot, S. Rosendahl, R. Kjøller, M. Paynot, J. Negrel et S. Gianinazzi. 1999. Endoproteolytic activities in pea roots inoculated with the arbuscular mycorrhizal fungus *Glomus mosseae* and/or *Aphanomyces euteiches* in relation to bioprotection. New Phytologist 142:517-529.

Slezack, S., E. Dumas-Gaudot, M. Paynot et S. Gianinazzi. 2000. Is a fully established arbuscular mycorrhizal symbiosis required for bioprotection of *Pisum sativum* roots against *Aphanomyces euteiches*? . Molecular Plant Microbe Interactions 13:238-241.

Smit, E., P. Leeflang, S. Gommans, J. van den Broek, S. van Mil et K. Wernars. 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Applied & Environmental Microbiology 67:2284-2291.

Smith, S.E. et D.J. Read. 1997. Mycorrhizal symbiosis. 2nd edition. San Diego: Academic Press.

Smith, S.E., S. Dickson et F.A. Smith. 2001. Nutrient transfer in arbuscular mycorrhizas: how are fungal and plant processes integrated? . Australian Journal of Plant Physiology 28:683-694.

Smith, S.E., F.A. Smith et I. Jakobsen. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiology 133:16-20.

Söderberg, K.H., P.A. Olsson et E. Bååth. 2002. Structure and activity of the bacterial community in the rhizosphere of different plant species and the effect of arbuscular mycorrhizal colonisation. FEMS Microbiology Ecology 40:223-231.

Sood, S.G. 2003. Chemotactic response of plant-growth-promoting bacteria towards roots of vesicular-arbuscular mycorrhizal tomato plants. FEMS Microbiology Ecology 45:219-227.

Spanu, P. et P. Bonfante-Fasolo. 1988. Cell-wall bound peroxydase activity in roots of mycorrhizal *Allium porrum*. New Phytologist 109:119-124.

Spanu, P., T. Boller, A. Ludwig, A. Wiemken, A. Faccio et P. Bonfante-Fasolo. 1989. Chitinases in roots of mycorrhizal *Allium porrum*: regulation and localization. Planta 177:447-455.

Spencer, P.A. et G.H.N. Towers. 1991. Restricted occurence of acetophenone signal compounds. Phytochemistry 30:2933-2937.

Srinath, J., D. Bagyaraj et B. Satyanarayana. 2003. Enhanced growth and nutrition of micropropagated *Ficus benjamina* to *Glomus mosseae* co-inoculated with *Trichoderma harzianum* and *Bacillus coagulans*. World Journal of Microbiology & Biotechnology 19:69-72.

St-Arnaud, M., C. Hamel, M. Caron et J.A. Fortin. 1994. Inhibition of *Pythium ultimum* in roots and growth substrate of mycorrhizal *Tagetes patula* colonized with *Glomus intraradice*. Canadian Journal of Plant Pathology 16:187-194.

St-Arnaud, M., C. Hamel, M. Caron et J.A. Fortin. 1995a. Endomycorhizes VA et sensibilité aux maladies: synthèse de la littérature et mécanismes d'interaction potentiels. Dans *La symbiose mycorhizienne* – État des connaissances (ed. Fortin, J.A., C. Charest et Y. Piché), pp.51-87. Éditions Orbis: Frelighsburg, Québec.

St-Arnaud, M., C. Hamel, B. Vimard, M. Caron et J.A. Fortin. 1995b. Altered growth of *Fusarium oxysporum* f. sp. *chrysanthemi* in an *in vitro* dual culture system with the vesicular arbuscular mycorrhizal fungus *Glomus intraradices* growing on *Daucus carota* transformed roots. Mycorrhiza 5:431-438.

St-Arnaud, M., C. Hamel, B. Vimard, M. Caron et J.A. Fortin. 1996. Enhanced hyphal growth and spore production of the arbuscular mycorrhizal *Glomus intraradices* in an *in vitro* system in the absence of host roots. Mycological Research 100:328-332.

St-Arnaud, M., C. Hamel, B. Vimard, M. Caron et J.A. Fortin. 1997. Inhibition of *Fusarium oxysporum* f. sp. *dianthi* in the non-VAM species *Dianthus caryophyllus* by co-culture with *Tagetes patula* companion plants colonized by *Glomus intraradices*. Canadian Journal of Botany 75: 998-1005.

St-Arnaud, M. et A. Elsen. 2005. Interaction of arbuscular mycorrhizal fungi with soil-borne pathogens and non-pathogenic rhizosphere micro-organisms. Dans *In vitro culture of mycorrhizas* (ed. S. Declerck, D.-G. Strullu et J. A. Fortin), pp. 217-231. Berlin Heidelberg: Springer-Verlag.

St-Arnaud, M. et V. Vujanovic. 2007. Effect of the arbuscular mycorrhizal symbiosis on plant diseases and pests. Dans *Mycorrhizae in crop production* (ed. C. Hamel et C. Plenchette), pp. 67-122. Binghampton, New York: Haworth Press.

Stachel, S.C., E. Messens, M. Van Montagu et P. Zambryski. 1985. Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in *Agrobacterium tumefaciens*. Nature 318:624-629.

Stackebrandt, E. et B.M. Goebel. 1994. A place for DNA-DNA reassociation and 16S rRNAsequence analysis in the present species definition in bacteriology. International Journal of Systematic Bacteriology 44:846-849.

Stackebrandt, E., W. Frederiksen, G.M. Garrity, P.A.D. Grimont, P. Kampfer, M.C.J. Maiden, X. Nesme, R. Rosselló-Mora, J. Swings, H.G. Truper, L. Vauterin, A.C. Ward et W.B. Whitman. 2002. Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. International Journal of Systematic & Evolutionary Microbiology 52:1043-1047.

Steinberg, P.D. et M.C. Rillig. 2003. Differential decomposition of arbuscular mycorrhizal fungal hyphae and glomalin. Soil Biology & Biochemistry 35:191-194.

Stevenson, P.C., D.E. Padgham et M.P. Haware. 1995. Root exudates associated with the resistance of four chickpea cultivars (*Cicer arietinum)* to two races of *Fusarium oxysporum* f. sp. *ciceri*. Plant Pathology 44:686-694.

Stevenson, F.J. et M.A. Cole. 1999. Cycles of soil: carbon, nitrogen, phosphorus, sulphur, micronutrients. Second Edition, John Wiley and Sons Inc.

Stewart, L., C. Hamel, R. Hogue et P. Moutoglis. 2005. Response of strawberry to inoculation with arbuscular mycorrhizal fungi under very high soil phosphorus conditions. Mycorrhiza 18:612-619.

Strack, D. et T. Fester. 2006. Isoprenoid metabolism and plastid reorganization in arbuscular mycorrhizal roots. New Phytologist 172:22-34.

Strack, D., T. Fester, B. Hause, W. Schliemann et M.H. Walter. 2003. Arbuscular mycorrhiza: biological, chemical, and molecular aspects. Journal of Chemical Ecology 29:1955-1979.

Ström, L., A.G. Owen, D.L. Godbold et D.L. Jones. 2002. Organic acid mediated P mobilization in the rhizosphere and uptake by maize roots. Soil Biology & Biochemistry 34:703-710.

Stumpe, M., J.G. Carsjens, I. Stenzel, C. Göbel, I. Lang, K. Pawlowski, B. Hause et I. Feussner. 2005. Lipid metabolism in arbuscular mycorrhizal roots of *Medicago truncatula*. Phytochemistry 66:781-791.

Subramanian, K.S. et C. Charest. 1998. Arbuscular mycorrhizae and nitrogen assimilation in maize after drought and recovery. Physiologia Plantarum 102:285-296.

Subramanian, K.S. et C. Charest. 1999. Acquisition of N by external hyphae of an arbuscular mycorrhizal fungus and its impact on physiological responses in maize under drought-stressed and well-watered conditions. Mycorrhiza 9:69-75.

Swofford. 2002. PAUP*: phylogenetic analysis using parsimony (and other methods), 4.0 Beta. Sinauer Associates, Sunderland, Massachusetts.

Talavera, M., K. Itou et T. Mizukubo. 2001. Reduction of nematode damage by root colonization with arbuscular mycorrhiza (*Glomus* spp.) in tomato-*Meloidogyne incognita* (Tylenchida: Meloidognidae) and carrot-*Pratylenchus penetrans* (Tylenchida: Pratylenchidae) pathosystems. Applied Entomology & Zoology 36:387-392.

Tanaka, Y. et K. Yano. 2005. Nitrogen delivery to maize via mycorrhizal hyphae depends on the form of N supplied. Plant Cell & Environment 28:1247-1254.

Tawaraya, K., M. Naito et T. Wagatsuma. 2006. Solubilization of insoluble inorganic phosphate by hyphal exudates of arbuscular mycorrhizal fungi. Journal of Plant Nutrition 29:657-665.

Taylor, T.N., W. Remy, H. Hass et H. Kerp. 1995. Fossil of arbuscular mycorrhizae from the early Devonian. Mycologia 87:560-573.

Taylor, J.M.W., A.D. Sutherland, K.E. Aidoo et N.A. Logan. 2005. Heat-stable toxin production by strains of *Bacillus cereus*, *Bacillus firmus*, *Bacillus megaterium*,

Bacillus simplex and *Bacillus licheniformis*. FEMS Microbiology Letters 242:313-317.

Theron, J. et T.E. Cloete. 2000. Molecular techniques for determining microbial diversity and community structure in natural environments. Critical Reviews in Microbiology 26:37-57.

Thygesen, K., J. Larsen et L. Bødker. 2004. Arbuscular mycorrhizal fungi reduce development of pea root-rot caused by *Aphanomyces euteiches* using oospores as pathogen inoculum. European Journal of Plant Pathology 110:411-419.

Tiedje, J.M., S. Asuming-Brempong, K. Nusslein, T.L. Marsh et S.J. Flynn. 1999. Opening the black box of soil microbial diversity. Applied Soil Ecology 13:109-122.

Toljander, J.F., V. Artursson, L.R. Paul, J.K. Jansson et R.D. Finlay. 2006. Attachment of different soil bacteria to arbuscular mycorrhizal fungal extraradical hyphae is determined by hyphal vitality and fungal species. FEMS Microbiology Letters 254:34-40.

Toro, M., R. Azcón et J.M. Barea. 1997. Improvement of arbuscular mycorrhiza development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (^{32}P) and nutrient cycling. Applied & Environmental Microbiology 63:4408-4412.

Torsvik, V., J. Goksøyr et F.L. Daae. 1990. High diversity in soil DNA bacteria. Applied and Environmental Microbiology 56:782-787.

Toussaint, J.P., M. St-Arnaud et C. Charest. 2004. Nitrogen transfer and assimilation between the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck & Smith and Ri T-DNA roots of *Daucus carota* L. in an *in vitro* compartmented system. Canadian Journal of Microbiology 50:251-260.

Trappe, J.M. 1987. Phylogenetic and ecologic aspects of mycotrophy in the Angiosperms from an evolutionary standpoint. Dans *Ecophysiology of VA Mycorrhizal plants*, (ed. Safir, G. R.), pp. 5-25. Boca Raton: Florida, CRC Press Inc.

Traquair, J.A. 1995. Fungal biocontrol of root diseases: endomycorrhizal suppression of Cylindrocarpon root rot. Canadian Journal of Botany 73:S89-S95.

Trotsenko, Y.A., E.G. Ivanova et N.V. Doronina. 2001. Aerobic methylotrophic bacteria as phytosymbionts. Microbiology 70:623-632.

Trotta, A., G.C. Varese, E. Gnavi, A. Fusconi, S. Sampo et G. Berta. 1996. Interactions between the soilborne root pathogen *Phytophthora nicotianae* var. *parasitica* and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. Plant & Soil 185:199-209.

Tuite J. 1969. Plant Pathological Methods. Burgess Publishing Company, Minneapolis, USA.

Uren, N.C. 2000. Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. Dans *The rhizosphere: biochemistry and organic substances at the soil-plant interface* (ed. Pinton, R. Z. Varanini et P.Nannipieri), New York: Marcel Dekker Inc.

Uren, N.C. et H.M. Reisenauer. 1988. The role of root exudates in nutrient acquisition. In *Advances in Plant Nutrition.* Vol 9.

Usha, K., A. Saxena et B. Singh. 2004. Rhizosphere dynamics influenced by arbuscular mycorrhizal fungus (*Glomus deserticola*) and related changes in leaf nutrient status and yield of Kinnow mandarin {King (*Citrus nobilis*) x Willow Leaf (*Citrus deliciosa*)}. Australian Journal of Agricultural Research 55:571-576.

van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken et I.R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69-72.

van West, P., A.A. Appiah et N.A.R. Gow. 2003. Advances in research on oomycete root pathogens. Physiological & Molecular Plant Pathology 62:99-113.

Vassilev, N., A. Medina, R. Azcón et M. Vassileva. 2006. Microbial solubilization of rock phosphate on media containing agro-industrial wastes and effect of the resulting products on plant growth and P uptake. Plant & Soil 287:77-84.

Vassileva, M., N. Vassilev et R. Azcón. 1998. Rock phosphate solubilization by *Aspergillus niger* on olive cake-based medium and its further application in a soil-plant system. World Journal of Microbiology & Biotechnology 14:281-284.

Vazquez, M.M., S. Cesar, R. Azcón et J.M. Barea. 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Applied Soil Ecology 15:261-272.

Vazquez, M.M., R. Azcón et J.M. Barea. 2001. Compatibility of a wild type and its genetically modified *Sinorhizobium* strain with two mycorrhizal fungi on *Medicago* species as affected by drought stress. Plant Science 161:347-358.

Versalovic, J., M. Schneider, F.J. de Bruijn et J.R. Lupsi. 1994. Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Methods in Molecular and Cellular Biology 5:25-40.

Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant & Soil 255:571-586.

Vestberg, A., S. Kukkonen, K. Saari, P. Parikka, J. Huttunen, L. Tainio, D. Devos, F. Weekers, C. Kevers, P. Thonart, M.C. Lemoine, C. Cordier, C. Alabouvette et S. Gianinazzi. 2004. Microbial inoculation for improving the growth and health of micropropagated strawberry. Applied Soil Ecology 27:243-258.

Viereck, N., P.E. Hansen et I. Jakobsen. 2004. Phosphate pool dynamics in the arbuscular mycorrhizal fungus *Glomus intraradices* studied by in vivo P-31 NMR spectroscopy. New Phytologist 162:783-794.

Vierheilig, H. et J. Ocampo. 1990. Role of root extract and volatile substances of non-host plants on vesicular-arbuscular mycorrhizal spore germination. Symbiosis 9:199-202.

Vierheilig, H., M. Alt, P. Mader, T. Boller et A. Wiemken. 1990. Spreading of *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus, across the rhizosphere of host and non-host plants. Soil Biology & Biochemistry 27:1113-1115.

Vierheilig, H., A.P. Coughlan, U. Wyss et Y. Piché. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Applied & Environmental Microbiology 64:5004-5007.

Vierheilig, H., H. Gagnon, D. Strack et W. Maier. 2000a. Accumulation of cyclohexenone derivatives in barley, wheat and maize roots in response to inoculation with different arbuscular mycorrhizal fungi. Mycorrhiza 9:291-293.

Vierheilig, H., J.M. García-Garrido, U. Wyss et Y. Piché. 2000b. Systemic suppression of mycorrhizal colonisation of barley roots already colonized by AM fungi. Soil Biology & Biochemistry 32:589-595.

Vierheilig, H. et Y. Piché. 2002. Signalling in arbuscular mycorrhiza: facts and hypotheses. Dans *Flavonoids in cell function* (ed. B. Bushlig et J. Manthey), pp. 23-39. New York: Kluwer Academic/Plenum Publishers

Vierheilig, H., S. Lerat et Y. Piché. 2003. Systemic inhibition of arbuscular mycorrhiza development by root exudates of cucumber plants colonized by *Glomus mosseae*. Mycorrhiza 13:167-170.

Vierheilig, H. 2004. Regulatory mechanisms during the plant-arbuscular mycorrhizal fungus interaction. Canadian Journal of Botany 82:1166-1176.

Vigo, C., J.R. Norman et J.E. Hooker. 2000. Biocontrol of the pathogen *Phytophthora parasitica* by arbuscular mycorrhizal fungi is a consequence of effects on infection loci. Plant Pathology 49:509-514.

Villegas, J. et J.A. Fortin. 2001. Phosphorus solubilization and pH changes as a result of the interactions between soil bacteria and arbuscular mycorrhizal fungi on a medium containing NH4+ as nitrogen source. Canadian Journal of Botany 79:865-870.

Vivas, A., A. Marulanda, J.M. Ruiz-Lozano, J.M. Barea et R. Azcón. 2003. Influence of a *Bacillus* sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG-induced drought stress. Mycorrhiza 13:249-256.

Von Broembsen, S.L. et J.W. Deacon. 1996. Effects of calcium on germination and further zoospore release from zoospore cysts of *Phytophthora parasitica*. Mycological Research 100:1498-1504.

von der Weid, I., V. Artursson, L. Seldin et J.K. Jansson. 2005. Antifungal and root surface colonization properties of GFP-tagged *Paenibacillus brasilensis* PB177. World Journal of Microbiology & Biotechnology 21:1591-1597.

Vujanovic, V., C. Hamel, E. Yergeau et M. St-Arnaud. 2006. Biodiversity and biogeography of *Fusarium* species from northeastern North American asparagus fields based on microbiological and molecular approaches. Microbial Ecology 51:242-255.

Walker, R., A.A. Powell et B. Seddon. 1998. Bacillus isolates from the spermosphere of peas and dwarf french beans with antifungal activity against *Botrytis cinerea* and Pythium species. Journal of Applied Microbiology 84:791-801.

Walley, F.L. et J.J. Germida. 1995. Estimating the viability of vesicular-arbuscular mycorrhizae fungal spores using tetrazolium salts as vital stains. Mycologia 87:273-279.

Wamberg, C., S. Christensen, I. Jakobsen, A.K. Müller et S.J. Sørensen. 2003. The mycorrhizal fungus (*Glomus intraradices*) affects microbial activity in the rhizosphere of pea plants (*Pisum sativum*). Soil Biology & Biochemistry 35:1349-1357.

Wang, B. et Y.-L. Qiu. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299-363.

Warburton, A.J. et J.W. Deacon. 1998. Transmembrane Ca2+ fluxes associated with zoospore encystment and cyst germination by the phytopathogen *Phytophthora parasitica*. Fungal Genetics & Biology 25:54-62.

Wayne, L.G., D.J. Brenner et R.R.O.A. Colwell. 1987. International Committee on Systematic Bacteriology. Report of the the ad hoc committee on reconciliation of approaches to bacterial systematics. International Journal of Systematic and Bacteriology 37:463-464.

Whipps, J.M. 1987. Carbon loss from the roots of tomato and pea seedlings grown in soil. Plant & Soil 103:95-100.

Whipps, J.M. 2001. Microbial interactions and biocontrol in the rhizosphere. Journal of Experimental Botany 52:487-511.

Whipps, J.M. 2004. Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Canadian Journal of Botany 82:1198-1227.

Whitelaw, M.A. 2000. Growth promotion of plants inoculated with phosphate-solubilizing fungi. Advances in Agronomy 69:99-151.

Witter, E. et A. Kanal. 1998. Characteristics of the soil microbial biomass in soils from a long-term field experiment with different levels of C input. Applied Soil Ecology 10:37-39.

Wright, S.F. et A. Upadhyaya. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant & Soil 198:97-107.

Wright, S.F., M. Frankesnyder, J.B. Morton et A. Upadhyaya. 1996. Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant & Soil 181:193-203.

Wright, S.F., J.L. Starr et I.C. Paltineanu. 1999. Changes in aggregate stability and concentration of glomalin during tillage management transition. Soil Science Society of America Journal 63:1825-1829.

Wu, Q.S. et R.X. Xia. 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. Journal of Plant Physiology 163:417-425.

Wyss, P., T. Boller et A. Wiemken. 1991. Phytoalexin response is elicited by a pathogen (*Rhizoctonia solani*) but not by a mycorrhizal fungus (*Glomus mosseae*) in soybean roots. Experientia 47:395-399.

Xavier, L.J.C. et J.J. Germida. 2003. Bacteria associated with *Glomus clarum* spores influence mycorrhizal activity. Soil Biology & Biochemistry 35:471-478.

Xu, C. et P.F. Morris. 1998. External calcium controls the developmental strategy of *Phytophthora sojae* cysts. Mycologia 90:269-275.

Yang, C.H., D.E. Crowley et J.A. Menge. 2001. 16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and *Phytophthora* infected avocado roots. FEMS Microbiology Ecology 35:129-136.

Yao, M.K., H. Desilets, M.T. Charles, R. Boulanger et R.J. Tweddell. 2003. Effect of mycorrhization on the accumulation of rishitin and solavetivone in potato plantlets challenged with *Rhizoctonia solani*. Mycorrhiza 13:333-336.

Yergeau E. Filion M. Vujanovic V. St-Arnaud M. 2005. A PCR-denaturing gradient gel electrophoresis approach to assess Fusarium diversity in asparagus. [Article] Journal of Microbiological Methods. 60(2):143-154, 2005

Yergeau, E., V. Vujanovic et M. St-Arnaud. 2006. Changes in communities of *Fusarium* and arbuscular-mycorrhizal fungi as related to different asparagus cultural factors. Microbial Ecology 52:104-113.

Zelenev, V., A. van Bruggen et A. Semenov. 2000. "BACWAVE," a spatial-temporal model for traveling waves of bacterial populations in response to a moving carbon source in soil. Microbial Ecology 40:260-272.

Zentmyer, G.A. 1970. Tactic responses of zoospores of *Phytophthora*. Dans *Root diseases and soil-borne pathogens* (ed. Toussoun, T. A., R.V. Bega et P.E. Nelson), Berkeley: University of California Press.

Zhang, Y., L.D. Guo et R.J. Liu. 2004. Survey of arbuscular mycorrhizal fungi in deforested and natural forest land in the subtropical region of Dujiangyan, southwest China. Plant &Soil 261:257-263.

Zhu, H.H. et Q. Zao. 2004. Localized and systemic increase of phenols in tomato roots induced by *Glomus versiforme* inhibits *Ralstonia solanacearum*. Journal of Phytopathology 152:537-542.

Annexe 1

Etude des relations entre la concentration des composés quantifiés dans les exsudats de racines de tomate transformées et la chémotaxie des zoospores du Phytophthora nicotianae, in vitro

Les acides aminés, certains sucres et acides organiques ont été quantifiés dans les exsudats (concentration 10X) de racines de tomate transformées par *l'Agrobacterium rhizogenes* et cultivées *in vitro* (cf. chapitre IV). La chémotaxie des zoospores du *Phytophthora nicotianae* vis-à-vis de ces exsudats a également été analysée, *in vitro*. Quatre types d'exsudats ont été testés : ceux provenant de racines colonisées par le *Glomus intraradices* et ceux provenant de racines non-mycorhiziennes, avec deux temps de prélèvement des exsudats, après 16 sem. et 24 sem. de croissance racinaire. Nous analysons ici les relations entre ces paramètres.

Matériels et Méthodes

La corrélation entre le nombre de zoospores présentes dans les capillaires emplis d'exsudats racinaires, à une concentration de 10× et la concentration de chacune des molécules quantifiées dans ces exsudats, ainsi que la corrélation entre les concentrations des différentes molécules entre elles et la régression multiple du nombre de zoospores en fonction de la quantité de chacune de ces molécules furent analysées en utilisant les procédures de Corrélation et de Régression séquentielle (stepwise) du logiciel SAS/STAT, version 9.1.3 du système SAS pour Windows (SAS Institute Inc. 2004).

Résultats

Les quantités de glucose (R=0,52) et de fructose (R=0,51) montraient une corrélation importante avec le nombre de zoospores présentes dans les capillaires emplis d'exsudats (Tableau X). Les quantités de thréonine (R=0,33), de proline (R=-0,25), d'alanine (R=0,24), d'acide oxalique (R=0,15), d'acide formique (R=0.13), de glycine (R=-0,13), d'asparagine (R=0,13), de serine (R=0,12) et de lysine (R=0,12) présentaient une corrélation également non-négligeable avec cette donnée. Les quantités des autres molécules présentaient une corrélation faible (R<0,1) avec le nombre de zoospores.

L'analyse de régression séquentielle s'est déroulée selon sept étapes. La quantité de glucose présente dans les exsudats (entrée lors de la première étape) est la valeur qui explique la plus grande proportion de la variabilité du nombre de zoospores présentes dans les capillaires (R^2 partiel=0,270) (Tableau XI). Les quantités d'alanine, de glycine, de proline, d'asparagine et de sérine expliquent la variation du nombre de zoospores selon une valeur de R^2 comprise entre 0,01 et 0,1. La quantité d'arginine a été incluse dans le modèle mais selon R^2=0,001. La quantité de proline a été incluse dans le modèle lors de la quatrième étape. La pente de la droite de régression entre le nombre de zoospores et la concentration des molécules prises en compte par le modèle étaient toujours significativement différentes de 0 (P<=0,005), sauf concernant la sérine (P=0.006) et l'arginine (P=0.122). Les autres molécules quantifiées n'ont pas été incluses dans le modèle de régression.

Tableau X. Corrélation entre le nombre de zoospores présentes dans les capillaires emplis d'exsudats de racines de tomate transformées et entre la concentration des composés quantifiés dans ce exsudats

	Coefficient de corrélation R								
	Fruc[1]	Gluc[2]	Ala[3]	Arg[3]	Asn[3]	Asp[3]	Glu[3]	Gln[3]	Gly[3]
Nb. Zoospores	0,51	0,56	0,24	0,06	0,13	0,01	-0,12	-0,06	-0,13
Sucres									
Fructose	1,00	0,85	0,16	-0,20	0,13	0,02	-0,21	-0,10	-0,10
Glucose	0,85	1,00	0,05	-0,31	-0,00	0,03	-0,28	-0,12	-0,22
Acides aminés									
Alanine	0,16	0,05	1,00	0,37	0,48	0,54	0,10	0,17	0,32
Arginine	-0,20	0,31	0,37	1,00	0,55	0,80	0,87	0,67	0,73
Asparagine	0,13	0,00	0,48	0,55	1,00	0,61	0,57	0,72	0,81
Ac. Aspartique	-0,06	-0,20	0,54	0,78	0,61	1,00	0,77	0,66	0,72
Glutamine	-0,10	-0,11	0,17	0,67	0,72	0,66	0,86	1,00	0,96
Ac. Glutamique	-0,21	-0,28	0,09	0,87	0,57	0,77	1,00	0,86	0,83
Glycine	-0,09	-0,21	0,69	0,39	0,43	0,47	0,16	0,16	0,29
Isoleucine	-0,07	-0,13	0,32	0,64	0,81	0,72	0,83	0,96	0,30
Leucine	0,01	-0,09	0,55	0,73	0,90	0,72	0,64	0,80	0,50
Lysine	0,30	0,07	0,38	0,37	0,89	0,44	0,37	0,56	0,66
Phenylalanine	0,00	-0,12	0,56	0,79	0,73	0,81	0,75	0,81	0,37
Proline	-0,34	-0,26	0,15	0,38	0,53	0,41	0,51	0,67	0,69
Sérine	0,08	-0,03	0,86	0,49	0,41	0,59	0,20	0,19	0,33
Thréonine	0,39	0,25	0,89	0,34	-0,69	0,56	0,16	0,26	0,43
Tryptophane	0,01	-0,06	0,20	0,72	0,72	0,68	0,87	0,94	0,12
Valine	-0,00	-0,13	0,78	0,55	0,79	0,66	0,40	0,51	0,68
Ac.organiques									
Ac. formique	0,40	0,20	0,18	-0,37	0,26	0,02	-0,48	-0,41	0,16
Ac. isocitrique	0,05	-0,21	0,11	0,05	0,02	0,01	-0,12	-0,16	0,14
Ac. succinique	0,02	-0,27	0,09	0,30	0,25	0,24	0,23	0,86	0,11
Ac. oxalique	0,36	0,01	0,26	-0,09	-0,02	-0,11	-0,29	-0,27	0,25

	Coefficient de corrélation R								
	Ile[3]	Leu[3]	Lys[3]	Phe[3]	Pro[3]	Ser[3]	Thr[3]	Trp[3]	Val[3]
Nb. Zoospores	-0,03	0,00	0,12	0,05	-0,25	0,12	0,33	0,06	0,00
Sucres									
Fructose	-0,08	0,01	0,30	0,01	-0,34	0,08	0,39	0,01	-0,00
Glucose	-0,13	-0,10	0,07	-0,13	-0,27	-0,03	0,25	-0,06	-0,13
Acides aminés									
Alanine	0,32	0,56	0,38	0,56	0,15	0,86	0,89	0,20	0,78
Arginine	0,73	0,64	0,37	0,79	0,38	0,49	0,34	0,72	0,55
Asparagine	0,81	0,90	0,89	0,74	0,53	0,41	0,69	0,72	0,79
Ac. Aspartique	0,72	0,71	0,44	0,81	0,41	0,19	0,56	0,68	0,66
Glutamine	0,96	0,80	0,56	0,81	0,66	0,66	0,26	0,94	0,51
Ac. Glutamique	0,83	0,63	0,37	0,75	0,51	0,20	0,16	0,86	0,40
Glycine	0,29	0,49	0,53	0,37	0,15	0,57	0,59	0,12	0,65
Isoleucine	1,00	0,91	0,66	0,90	0,69	0,33	0,43	0,94	0,68
Leucine	0,91	1,00	0,78	0,88	0,69	0,54	0,68	0,77	0,89
Lysine	0,66	0,78	1,00	0,62	0,34	0,31	0,63	0,60	0,75
Phenylalanine	0,89	0,88	0,62	1,00	0,52	0,64	0,63	0,85	0,89
Proline	0,69	0,69	0,34	0,52	1,00	0,11	0,15	0,55	0,62
Sérine	0,33	0,54	0,31	0,64	0,11	1,00	0,80	0,19	0,71
Thréonine	0,43	0,68	0,63	0,63	0,15	0,79	1,00	0,33	0,80
Tryptophane	0,94	0,77	0,60	0,85	0,55	0,19	0,33	1,00	0,49
Valine	0,68	0,89	0,68	0,75	0,62	0,71	0,80	0,48	1,00
Ac. organiques									
Ac. formique	-0,38	-0,30	-0,07	-0,29	-0,43	-0,01	0,09	-0,33	-0,14
Ac. isocitrique	-0,05	0,05	0,29	0,12	-0,12	0,12	0,08	-0,02	0,13
Ac. succinique	0,13	0,14	0,29	0,17	-0,01	-0,03	0,06	0,20	0,14
Ac. oxalique	-0,19	-0,09	0,19	-0,06	-0,35	0,08	0,18	-0,14	0,05

	Coefficient de corrélation R			
	Ac. Formique	Ac. Isocitrique	Ac. Succinique	Ac. Oxalique
Nb. Zoospores	0,13	-0,05	0,05	0,15
Fructose	0,40	0,05	0,02	0,36
Glucose	0,20	-0,21	-0,27	0,01
Acides aminés				
Alanine	0,18	0,11	0,09	0,26
Arginine	-0,38	0,53	0,30	-0,09
Asparagine	-0,26	0,02	0,25	-0,02
Ac. Aspartique	-0,26	0,01	0,56	-0,11
Glutamine	-0,41	-0,16	0,08	-0,27
Ac. Glutamique	-0,48	-0,11	0,23	-0,29
Glycine	0,16	0,14	0,11	0,25
Isoleucine	-0,38	-0,05	0,14	-0,19
Leucine	-0,30	0,05	0,14	-0,09
Lysine	-0,07	0,29	0,29	0,20
Phenylalanine	-0,29	0,12	0,17	-0,06
Proline	-0,43	-0,18	-0,01	-0,35
Sérine	-0,01	0,11	0,80	0,08
Thréonine	0,09	0,08	0,06	0,18
Tryptophane	-0,33	-0,02	0,20	0,14
Valine	-0,15	0,13	0,15	0,04
Ac. organiques				
Ac. formique	1,00	0,26	0,21	0,84
Ac. isocitrique	0,26	1,00	0,65	0,60
Ac. succinique	0,21	0,65	1,00	0,60
Ac. oxalique	0,84	0,60	0,60	1,00

[1] Fructose.

[2] Glucose.

[3] Acides aminés selon le code trois lettres du système international.

Tableau XI. Analyse de régression multiple du nombre de zoospores présentes dans les capillaires emplis d'exsudats de racines de tomate transformées en fonction de la concentration des composés présents dans ces exsudats

Composé	Etape[1]	R^2 partiel [2]	R^2 du modèle [3]	P [4]
Glucose	1	0.270	0.270	<0.001
Alanine	2	0.046	0.316	0.001
Glycine	3	0.066	0.382	<0.001
Proline	4	0.029	0.411	0.005
Asparagine	5	0.041	0.452	<0.001
Serine	6	0.025	0.477	0.006
Arginine	7	0.001	0.485	0.122

[1] Ordre d'entrée des composés dans le modèle.

[2] Coefficient de détermination de chaque composé expliquant le nombre de zoospores présentes dans les capillaires.

[3] Coefficient de détermination du composé et des précédents intégrés dans le modèle, permettant d'expliquer le nombre de zoospores présentes dans les capillaires.

[4] Taux de probabilité de corrélation entre le composé pris en compte par le modèle et le nombre de zoospores présentes dans les capillaires.

Discussion

Les quantités de fructose et de glucose sont fortement corrélées au nombre de zoospores présents dans les capillaires. Pourtant, le fructose n'a pas été pris en compte par le modèle de régression. Ceci est dû à la forte corrélation entre les quantités de ces deux molécules (R=0,85) et à la procédure de régression séquentielle qui ne retient que les variables qui augmentent la proportion de variabilité expliquée par le modèle. Aussi le glucose présente une régression positive et très significative avec le nombre de zoospores. Dukes et Apple (1961) ont montré que les zoospores du *P. nicotianae* étaient attirées par des sucres (le sucrose, le dextrose, le fructose, le rhamnose et le maltose) lors de biotest utilisant des solutions de chacune de ces molécules. Cependant, les concentrations de glucose et de fructose n'étaient pas significativement différentes entre les différents traitements (âge et colonisation par le *G. intraradices*) dans notre étude. Même si les zoospores sont attirées par les sucres, cette attraction ne permet pas d'expliquer la chémotaxie différentielle des zoospores vers les exsudats de racines mycorhiziennes et non-mycorhiziennes. D'autres molécules présentes dans les exsudats de notre étude modifieraient les relations entre le nombre de zoospores et les quantités de glucose et de fructose. Les exsudats de racines colonisées et collectés après 24 semaines pourraient notamment contenir des molécules répulsives puisqu'ils repoussent les zoospores.

Les corrélations non négligeables observées entre le nombre de zoospores comptabilisées dans les capillaire et les quantités de thréonine, d'alanine, d'acide oxalique, d'acide formique et de lysine ne semblent pas non plus corrélées avec l'attraction différentielle des zoospores par les exsudats de racines mycorhiziennes et

non-mycorhiziennes que nous avons observées. En effet, aucun effet significatif de l'inoculation avec le *G. intraradices* ou de l'âge des racines n'était observé sur les concentrations de ces molécules. La thréonine n'était pas incluse dans le modèle de régression vraisemblablement à cause de sa forte corrélation avec l'alanine (R^2=0,88) et la sérine (R^2=0,78).

La quantité de proline était significativement plus élevée dans les exsudats de racines colonisées et collectés après 24 semaines en comparaison à la quantité présente dans les exsudats de racines non-colonisées et/ou collectées après 16 semaines de culture. Cet acide aminé présente une corrélation non-negligeable et une régression négative significative avec le nombre de zoospores et a été incluse dans le modèle de régression. Ainsi le nombre de zoospores serait négativement corrélé à la quantité de proline dont l'accumulation dans les exsudats de racines colonisées et collectés après 24 semaines de croissance repousserait les zoospores. Cette répulsion réduirait le nombre de zoospores du *P. nicotianae* capables d'entrer en contact avec les racines hôtes lorsque celles-ci sont colonisées par un champignon mycorhizien à arbuscules. Cette faible capacité d'infection de cet agent pathogène permettrait à la plante d'activer ses réactions de défense efficacement réduisant ainsi la prolifération intraracinaire de l'agent pathogène. Ceci permettrait d'expliquer le biocontrôle observé chez des plantes de tomate colonisés par le *G. intraradices* (cf. chapitre V) ou par le *G. mosseae* (cf. chapitre V; Cordier et al., 1996; Pozo et al., 2002a; Trotta et al., 1996; Vigo et al., 2000).

Annexe 2

Système compartimenté décrit dans le chapitre V

Les différentes étapes suivies lors de l'expérience exposée dans le chapitre V sont ici détaillées. Le système compartimenté ici décrit était utilisé afin de vérifier, en conditions non-axéniques, en sol, l'hypothèse que le biocontrôle exercé par les champignons mycorhiziens à arbuscules sur la maladie provoquée par le *Phytophthora nicotianae* infectant des plants de tomate est régi par la modification des exsudats racinaires. Les systèmes compartimentés sont représentés avec une vue de haut.

L'expérience présentait 12 traitements : G-E-P- , GiE-P-, GmE-P-, G-E^{G-}P-, G-E^{Gi}P-, G-E^{Gm}P-, G-E-P+ , GiE-P+, GmE-P+, G-E^{G-}P+, G-E^{Gi}P+, E-E^{Gm}P+, où :

G- signifie plant non-inoculé avec un champignon mycorhizien à arbuscules ;

Gi, signifie plant inoculé avec le *Glomus intraradices* ;

Gm, signifie plant inoculé avec le *G. mosseae* ;

E-, signifie plant plant arrosé avec de l'eau pure ;

E^{G-}, signifie plant recevant des exsudats de plants de tomate non-mycorrhizés ;

E^{Gi}, signifie plant recevant des exsudats de plants de tomate colonisés par le *G. intraradices* ;

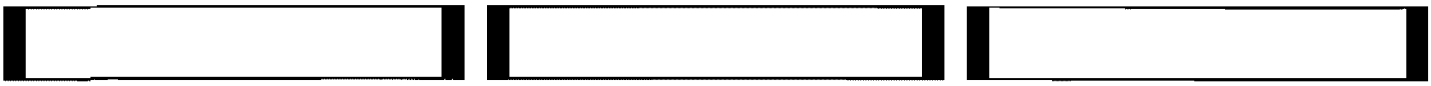
E^{Gm}, signifie plant recevant des exsudats de plants de tomate colonisés par le *G. mosseae* ;

P-, le passage du *Phytophthora nicotianae* vers le plant de tomate est impossible à partir du compartiment inoculé avec cet agent pathogène ;

P+, la colonisation du plant de tomate par le *P. nicotianae* est possible à partir du compartiment inoculé avec cet agent pathogène.

Les membranes en nylon (dont les pores présentaient 60 µm de diamètre et ainsi étaient perméables aux microorganismes du sol mais pas aux racines) avaient été préalablement collées sur des plaquettes de type C (Fig. 9). Ces plaquettes étaient insérées entre les sous-unités de type A et/ou B (Fig. 9) pour compartimenter le sol. Les systèmes étaient maintenus de part et d'autres par des plaquettes, des vis et des serres de menuisier. Ces derniers éléments ne sont pas indiqués sur les schémas pour une meilleure visibilité.

Etape 1 : Colonisation de plants de poireaux par le *G. mosseae* ou le *G. intraradices*



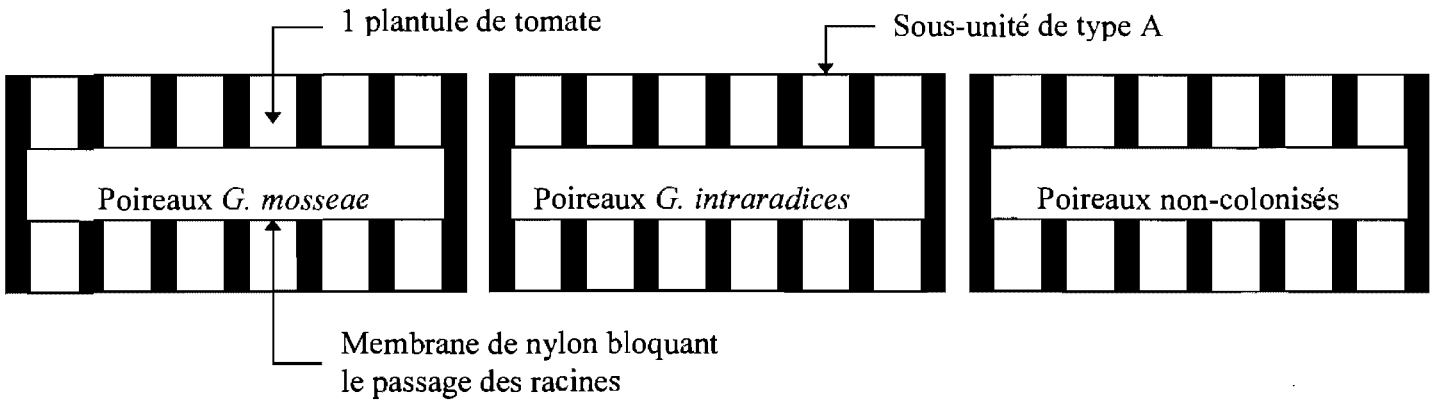
6 plants de poireaux + spores du *G. mosseae* recueillies par culture en sol puis stérilisées en surface

6 plants de poireaux + spores du *G. intraradices* recueillies après culture *in vitro*

6 plants de poireaux

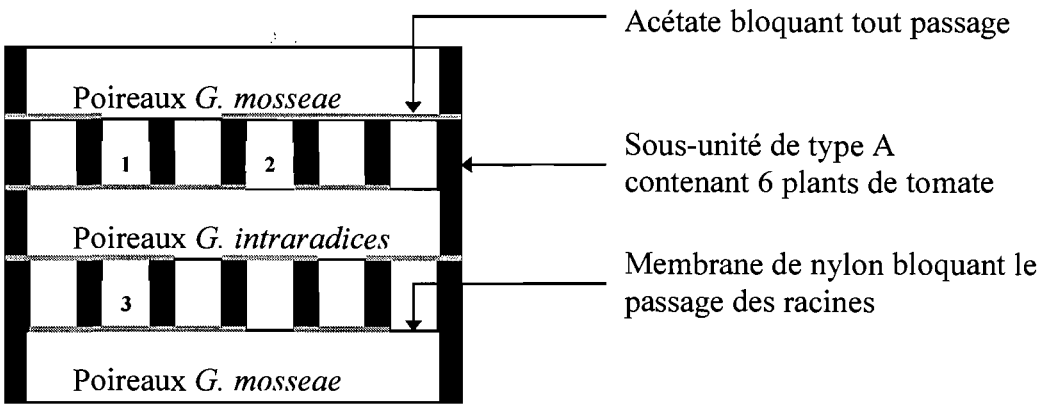
Utilisation de sous-unités de type B (Fig. 9).

Etape 2 : Production d'exsudats de racines colonisées par le *G. mosseae*, le *G. intraradices* ou non-colonisées



Après 5 semaines, les plants de tomate étaient recueillis afin de collecter des exsudats par incubation des racines dans de l'eau stérile.

Etape 3 : Inoculation avec les champignons mycorhiziens à arbuscules : le *Glomus intraradices* ou le *G. mosseae*



1 : Plant de tomate colonisé par le *G. mosseae* (traitement Gm E-)

2 : Plant de tomate colonisé par le *G. intraradices* (traitement Gi E-)

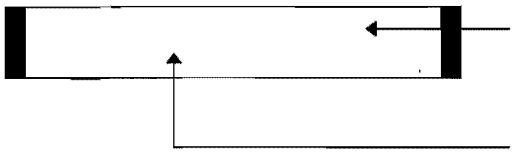
3 : Plant de tomate non-colonisé (traitement G- ; E- ou E+)

Cette étape avait une durée de trois semaines. Un tel système correspondait à un bloc expérimental composé des 12 traitements précisés ci-haut.

Les acétates avaient été préalablement collés de chaque côté des sous-unités de type A, avec de la colle de silicone, avant l'inoculation du sol et des plantules. Ils avaient été sectionnés au niveau d'un compartiment, pour permettre le passage des hyphes mycorhiziennes vers les racines de tomate de traitement Gm ou Gi.

Concernant le traitement avec ajout d'exsudats (E+), les plantules étaient arrosées quotidiennement avec des exsudats de plants de tomate colonisés par soit le *G. intraradices*, soit le *G. mosseae* ou non-colonisés. L'ajout d'exsudats était également effectué durant l'étape 4. Les autres plantules (traitement E-) recevaient une quantité équivalente d'eau pure stérilisée.

* Préparation de l'inoculum du *Phytophthora nicotianae*

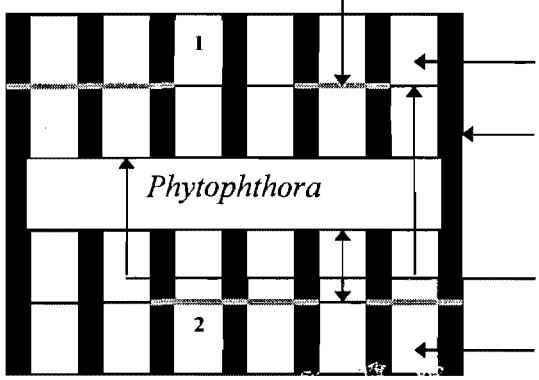


6 plantules de tomate (âgées d'une semaine)

Inoculation du mycélium du *P. nicotianae* à la surface du sol

Utilisation de sous-unités de type B. Après deux semaines, les plantules étaient sectionnées au niveau de la base de leur tige et la sous-unité était accotée à des plants préalablement inoculés avec les champignons mycorhiziens.

Etape 4 : Les plants de tomate sont accotés au compartiment central inoculé avec le *P. nicotianae*



Acétate bloquant tout passage

1 plant de tomate

Compartiment intermédiaire contenant du substrat de culture seulement

Membranes de nylon bloquant le passage des racines

1 plant de tomate

1 : Plant de tomate inoculé avec le *P. nicotianae* (traitement P+)

2 : Plant de tomate non-inoculé avec le *P. nicotianae* (traitement P-)

Les acétates des sous-unités A étaient préalablement remplacés. L'acétate était sectionné au niveau d'un compartiment pour permettre le passage du *P. nicotianae* vers le plant de tomate adjacent (traitement P+).

Etape 5 :

Après trois semaines, les plants de tomate étaient recueillis afin de mesurer leur poids sec aérien, poids frais racinaire, taux de nécrose racinaire, taux de colonisation par les champignons mycorhiziens et biomasse intraracinaire du *P. nicotianae* par test ELISA. Le sol, duquel toute racine avait été prélevée, était placé immédiatement à -20°C, afin d'en extraire l'ADN et effectuer un DGGE permettant l'analyse de la structure de la communauté bactérienne de la rhizosphère de tomate, tel que décrit dans le chapitre VI.